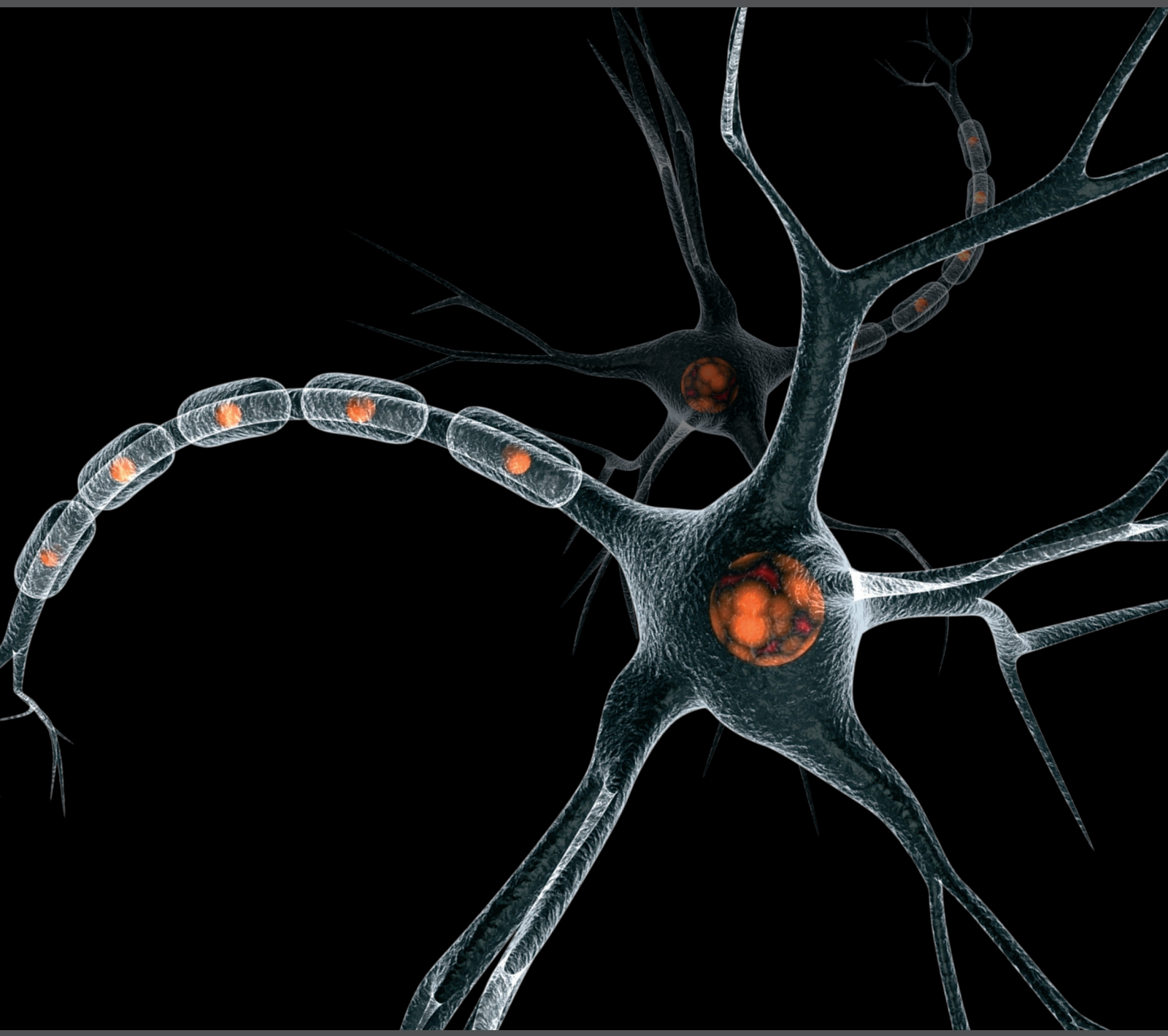
Neural Plasticity

Ion Channel Modulation in Spinal/Trigeminal Synaptic Plasticity

Guest Editors: Dong-ho Youn, Gábor Gerber, and William A. Sather



Ion Channel Modulation in Spinal/Trigeminal Synaptic Plasticity

Neural Plasticity

Ion Channel Modulation in Spinal/Trigeminal Synaptic Plasticity

Guest Editors: Dong-ho Youn, Gábor Gerber,
and William A. Sather

Editorial Board

Hindawi Publishing Corporation
Neural Plasticity
Volume 2014, Article ID 345841, 3 pages
http://dx.doi.org/10.1155/2014/345841

Editorial

Ion Channel Modulation in Spinal/Trigeminal Synaptic Plasticity

Dong-ho Youn,[1] Gábor Gerber,[2] and William A. Sather[3]

[1] *Department of Oral Physiology, School of Dentistry, Kyungpook National University, 188-1 Samduck-2, Chung-gu, Daegu 700-412, Republic of Korea*
[2] *Department of Anatomy, Histology and Embryology, Semmelweis University, Tüzoltó utca 58, Budapest 1450, Hungary*
[3] *Department of Pharmacology, University of Colorado School of Medicine, Mail Stop 8315, 12800 E. 19th Avenue, P18-7104, Aurora, CO 80045, USA*

Correspondence should be addressed to Dong-ho Youn; dyoun@knu.ac.kr

Received 10 February 2014; Accepted 10 February 2014; Published 12 March 2014

The sensory experience of pain provides an early warning sign to protect the body from tissue injury. Although pain is a straightforward symptom found in virtually every field of medicine, the cellular and molecular bases underlying the sensation of pain are complex and involve diverse mechanisms in a variety of areas of the brain and spinal cord. In 1965, Melzack and Wall proposed the gate control theory of pain [1], which posited the role of the dorsal horn (DH) of the spinal cord as a site for gating and brain control of pain. In the decades since then, research on pain mechanisms in the spinal DH has exploded, as has research in the spinal trigeminal nucleus (Vsp) for orofacial and head pain. The spinal DH has a laminated structure, consisting of superficial (laminae I and II) and deep (laminae III–VI) layers. The Vsp is, in a rostrocaudal sense, divided into oralis, interpolaris, and caudalis subnuclei. The caudalis of Vsp has particularly captured the attention of orofacial pain researchers, as this area is analogous to the DH of the spinal cord. Neurons in the spinal DH and Vsp form synapses with primary afferent fibers from peripheral and trigeminal ganglia, descending fibers from higher brain areas, and axonal fibers arising from other local neurons. The neurons of the spinal DH and Vsp employ a wide variety of ligand- and voltage-gated ion channels to support synaptic transmission, neuronal excitability, and proper relay of sensory and nociceptive information. Short-term and long-term modulation of the properties of these ion channels provide mechanisms for neural plasticity and changes in gene expression that can in turn lead to structural modification of neurons in pain pathways of the spinal DH and Vsp. The modulation of ion channels includes changes in their phosphorylation/dephosphorylation state, the composition of subunits contributing to channel formation, and interactions with other signaling molecules and scaffolding proteins that target modulators to channels. In this special issue, we focus on the roles of ion channel modulation in spinal/trigeminal mechanisms of neural plasticity that contribute to chronic pain. Advances in understanding mechanisms of ion channel modulation are expected to lead to improved approaches for management of chronic pain.

The first paper "*Ionotropic glutamate receptors and voltage-gated Ca^{2+} channels in long-term potentiation of spinal dorsal horn synapses and pain hypersensitivity*" in this issue introduces the anatomical and synaptic organization of the spinal DH. Although the anatomical location of the spinal DH is easily identified in transverse or parasagittal sections, identifying specific neuronal types and synaptic circuitry is challenging for two reasons. The first dilemma is that neurons in this area are not arranged in a precisely-organized structure; instead, neurons of any given functional subtype are scattered in a seemingly random way within the DH. Determining the pattern of synaptic connections between functional neuronal subtypes is as a consequence exceedingly difficult. The second challenge is that DH neurons are multimodal, meaning that any given subpopulation of DH neurons is involved in conveying more than one form of sensory information. Among the sensory modalities supported by

DH neurons are mechanical touch, pinch, thermal heat, and cold. In this issue's first paper, the authors synthesize findings in the recent literature to present an integrated model of the synaptic organization that supports pain processing in the spinal DH. The model also incorporates the synaptic circuitry suggested in the gate control theory of pain [1]. The paper proceeds to discuss recent published work regarding the contributions in spinal DH of ionotropic glutamate receptors (ion channels) and voltage-gated Ca^{2+} channels (VGCCs) to both long-term potentiation (LTP), an increase in the strength of synaptic transmission, and/or pain hypersensitivity. Consideration of the subtypes in both of these groups of ion channels distinguishes the differential contributions made by each type of ion channel to LTP and pain.

Next, A-R. Park et al. "*Dual effect of exogenous nitric oxide on neuronal excitability in rat substantia gelatinosa neurons*" report a remarkable effect of nitric oxide on the excitability of substantia gelatinosa (SG) neurons located in lamina II of the spinal DH. The directionality of the effect of nitric oxide is concentration-dependent: 10 μM sodium nitroprusside, a nitric oxide donor, causes depolarization of neurons, but 1 mM causes hyperpolarization. Both effects are mediated by soluble guanylyl cyclases. However, the hyperpolarizing effect of nitric oxide involves activation of various types of K^+ channels, while the depolarizing effect involves activation of certain types of Ca^{2+} channels. These findings may correlate with the complex effect of nitric oxide on pain behaviors, that is, a concentration-dependent switch between analgesic and hyperalgesic actions of nitric oxide.

T. T. H. Nguyen et al. "*Activation of glycine and extrasynaptic $GABA_A$ receptors by taurine on the substantia gelatinosa neurons of the trigeminal subnucleus caudalis*" report an effect of the free amino acid taurine, present at a high concentration in the brain, on the excitability of SG neurons located in the caudalis of Vsp. These authors report that taurine action on SG neurons of Vsp is mediated by glycine and $GABA_A$ receptors, but owing to the high concentration of chloride ions they employed in their recording pipettes, taurine application caused neuronal depolarization. With a lower, physiological level of internal chloride, taurine will exert an inhibitory action on neuronal excitability, which predicts an antinociceptive action of taurine on orofacial pain behaviors.

Kwi-H. Choi et al. "*Presynaptic glycine receptors increase GABAergic neurotransmission in rat periaqueductal gray neurons*" report that the periaqueductal gray (PAG) plays a role in the regulation of pain transmission via a descending inhibitory pathway to the spinal DH, thereby participating in the system postulated in the gate control theory. In this study, mechanically dissociated PAG neurons were voltage-clamped and spontaneous excitatory postsynaptic currents (EPSCs) were recorded. The axonal terminals, but not cell bodies, of presynaptic neurons remained attached to the neurons recorded, ruling out any contribution to the experimental results from presynaptic cell bodies. Using this method, the authors report facilitation by glycine of glutamate release, an effect that is mediated by glycine receptors and by voltage-gated Na^+ and Ca^{2+} channels. Facilitation of glutamate release by glycine may be due to glycine receptor-mediated depolarization of presynaptic terminals, an action which relies upon the high concentration of chloride ions found within the presynaptic terminals. Because facilitation of excitatory, glutamatergic synaptic input to PAG neurons is expected to increase activity in the descending inhibitory pathway from PAG to the spinal DH, the results of this study suggest glycine-induced facilitation of PAG output could suppress the sensation of pain.

In the final paper in this special issue, E. V. Khomula et al. "*Nociceptive neurons differentially express fast and slow T-type Ca^{2+} currents in different types of diabetes neuropathy*" report that two different kinds of T-type Ca^{2+} currents are present in isolectin B4 (IB4)-positive neurons of dorsal root ganglia (DRG). These neurons are small-diameter DRG neurons and respond to capsaicin application, suggesting that they are nonpeptidergic, C-type nociceptive neurons that are involved in the sensation of thermal pain. Based on measurements of the inactivation time constant ($\tau_{0.5}$) for T-type Ca^{2+} currents, IB4-positive neurons could be divided into two groups: ~70% of IB4-positive neurons exhibited fast-inactivating T-type current ($\tau_{0.5} < 50$ ms), and ~30% of IB4-positive neurons exhibited slow-inactivating T-current ($\tau_{0.5} > 50$ ms). Among T-type Ca^{2+} channel isoforms, the $Ca_V3.2$ isoform is more sensitive to block by Ni^{2+}, a property exploited by these authors to test the relative contribution of this isoform to fast or slow T-type currents. The authors found that fast-inactivating T-currents are more sensitive to Ni^+ than are the slow-inactivating T-currents, suggesting that fast-inactivating T-currents are carried by $Ca_V3.2$ channels. The work was extended to investigate the role of fast- and slow-inactivating T-currents in hyperalgesia, using streptozotocin-induced diabetic rats that displayed neuropathic hyperalgesia. Whereas 30% of the IB4-positive DRG neurons obtained from control animals exhibited slow-inactivating T-current, none of the IB4-positive neurons from the hyperalgesic rats possessed slow-inactivating T-type current; instead, IB4-positive neurons from hyperalgesic rats possessed exclusively fast-inactivating T-current and at increased density. Moreover, a depolarizing shift of steady-state inactivation of fast T-type current was found in IB4-positive neurons from hyperalgesic rats. Thus, this study suggests that a change in the functional properties of ion channels may cause a pathological state such as neuropathic pain.

This special issue highlights the importance of ion channel modulation in neuronal excitability, synaptic transmission, and synaptic plasticity of spinal DH and Vsp neurons, and it provides examples of ion channel modulation by chemicals and etiological factors. We hope that this issue will inspire further studies regarding various aspects of ion channel modulation in the spinal/trigeminal areas and other brain areas involved in pain transmission, thereby revealing new targets for pain treatment.

Acknowledgment

This study was supported by the Basic Science Research Program of the NRF of Korea, funded by the MEST (2008-0061215).

Dong-ho Youn
Gábor Gerber
William A. Sather

References

[1] R. Melzack and P. D. Wall, "Pain mechanisms: a new theory," *Science*, vol. 150, no. 3699, pp. 971–979, 1965.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2014, Article ID 938235, 12 pages
http://dx.doi.org/10.1155/2014/938235

Research Article

Nociceptive Neurons Differentially Express Fast and Slow T-Type Ca^{2+} Currents in Different Types of Diabetic Neuropathy

Eugen V. Khomula,[1,2] Anya L. Borisyuk,[2] Viacheslav Y. Viatchenko-Karpinski,[2] Andrea Briede,[2] Pavel V. Belan,[1,2] and Nana V. Voitenko[1,2]

[1] *International Center of Molecular Physiology of National Academy of Sciences of Ukraine, 4 Bogomoletz Street., Kyiv 01024, Ukraine*
[2] *State Key Laboratory of Molecular and Cellular Biology, Bogomoletz Institute of Physiology of National Academy of Sciences of Ukraine, 4 Bogomoletz Street, Kyiv 01024, Ukraine*

Correspondence should be addressed to Nana V. Voitenko; nana@biph.kiev.ua

Received 3 November 2013; Revised 24 December 2013; Accepted 30 December 2013; Published 18 February 2014

Academic Editor: Dong-ho Youn

T-type Ca^{2+} channels are known as important participants of nociception and their remodeling contributes to diabetes-induced alterations of pain sensation. In this work we have established that about 30% of rat nonpeptidergic thermal C-type nociceptive (NTCN) neurons of segments L4–L6 express a slow T-type Ca^{2+} current (T-current) while a fast T-current is expressed in the other 70% of these neurons. Streptozotocin-induced diabetes in young rats resulted in thermal hyperalgesia, hypoalgesia, or normalgesia 5-6 weeks after the induction. Our results show that NTCN neurons obtained from hyperalgesic animals do not express the slow T-current. Meanwhile, the fraction of neurons expressing the slow T-current did not significantly change in the hypo- and normalgesic diabetic groups. Moreover, the peak current density of fast T-current was significantly increased only in the neurons of hyperalgesic group. In contrast, the peak current density of slow T-current was significantly decreased in the hypo- and normalgesic groups. Experimental diabetes also resulted in a depolarizing shift of steady-state inactivation of fast T-current in the hyperalgesic group and slow T-current in the hypo- and normalgesic groups. We suggest that the observed changes may contribute to expression of different types of peripheral diabetic neuropathy occurring during the development of diabetes mellitus.

1. Introduction

Peripheral diabetic neuropathy (PDN), being one of the most frequent and troublesome complications of diabetes mellitus [1], is often accompanied with various pain syndromes [2–5]. Impairment of Ca^{2+} homeostasis [6–9] and remodeling of voltage- and ligand-gated ion channels [10–12] in nociceptive neurons under PDN have been implicated in altered nociception. Low voltage activated (LVA) T-type calcium channels (T-channels) [13], directly participating in cellular excitability as well as in intracellular calcium signaling, are crucially involved in both acute [14–19] and neuropathic pain [20–24]. It has been established that primary sensory neurons mainly express T-channels of the $Ca_v3.2$ subtype [17, 25, 26]. This subtype mediates a major part of LVA Ca^{2+} current (T-current) although other T-channel subtypes are also present in these neurons and may potentially contribute to the LVA current [17, 20, 25, 27]. Moreover, C-fiber nociceptors seem to be heterogeneous regarding amplitudes, pharmacology, and biophysical properties of T-current [28–30] and might be divided into two subclasses correspondingly expressing fast or slow T-current [28]. Despite these findings, a lot of studies proving the importance of T-channels for nociception do not distinguish between C-fiber nociceptors expressing fast and slow T-currents within populations of small and medium size nociceptive neurons. Differential remodeling of fast and slow T-currents in IB_4-positive capsaicin-sensitive small-sized DRG neurons [31], which are considered nonpeptidergic thermal C-type nociceptors (NTCN) [32], is of particular interest because of the strong involvement of these neurons in thermal pain sensitivity [33] and neuropathic pain [34, 35]. Recently it has been shown that in rats with streptozotocin- (STZ-) induced diabetes, the classical model of diabetes type 1 [4, 5], remodeling of T-channels in the NTCN neurons, was

PDN type specific with substantial differences in a case of the thermal hyperalgesia *versus* norm- or hypoalgesia [27].

Here we have used rats with thermal hyper-, hypo-, and normalgesia at the same age and duration of STZ-induced diabetes to determine PDN-type-specific remodeling of T-channels underlying fast and slow LVA Ca^{2+} currents in NTCN neurons.

2. Materials and Methods

2.1. Experimental Animals. All experimental protocols were approved by the Animal Care and Use Committee of the Bogomoletz Institute of Physiology (Kyiv, Ukraine) and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize animal suffering and the number of animals used.

2.2. Induction of Experimental Diabetes. We used a well-established model of streptozotocin (STZ) injections to induce diabetic neuropathy in young male Wistar rats (30–50 g, 21–23 days old) [5, 27]. Experimental diabetes was induced in rats by a single i.p. injection of STZ solution (80 mg/kg, i.p.). Blood glucose levels were checked on the third day after injection (to verify diabetes onset) and just before electrophysiological experiments (6-7 weeks after injections), using a blood glucometer (Accu-Chek Active; Roche Diagnostics, Indianapolis, IN, USA). Rats with values of >270 mg/dL (15 mM) were considered hyperglycemic.

2.3. Assessment of Thermal Nociception (Behavioral Experiments). Nociceptive responses to thermal stimulation were measured by the Hargreaves' method [27, 36] using a paw thermal stimulation system (Plantar Test, Ugo Basile, Italy) for the measurement of paw withdrawal latency (PWL). PWL was recorded for each tested rat as a mean of 10 measurements with 5 min interval alternating left and right hind paws.

2.4. Preparation of Dorsal Root Ganglia (DRG) Neurons. We prepared dissociated DRG cells and used them within 6–8 h for whole-cell recordings as described previously [27]. In brief, L4 and L5 DRGs were incubated in a Tyrode's solution containing 140 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES, adjusted to pH 7.4 with NaOH and supplemented with 1 mg/mL protease Type XIV (Sigma) and 0.5 mg/mL collagenase Type I (Worthington Biochemical Corporation) for 18–20 min at 35°C. Following incubation, ganglia were rinsed and dissociated by trituration with glass pipettes. Isolated neurons were plated onto an uncoated glass coverslip. All following experiments were done at room temperature.

2.5. IB4 Labeling and Imaging. Cells were incubated in Tyrode's solution supplemented with 10 μg/mL isolectin B4 (IB4) conjugated to Alexa Fluor 568 dye (Invitrogen) in the dark for 10–12 min [27]. Cells were visualized using a standard Rhodamine Filter Set (Chroma Technology, USA) installed in TILL Photonics wide-field imaging system (TILL Photonics, Gräfelfing, Germany) based on an inverted microscope (IX71, Olympus) and containing a monochromator Polychrome V and Imago CCD camera both controlled by TILLvision software (TILL Photonics). Fluorescent images were captured via an oil immersion objective (40x UV, NA 1.35; Olympus) under standardized settings from 15 to 20 randomly selected small DRG cells on each dish before any electrophysiological recordings during the first 15 min of each experiment. The mean intensity of halo of IB4 staining around the neuronal plasma membrane was determined for each neuron. The relative intensity was calculated separately for neurons of each coverslip. The 0 and 100% intensity values for a particular coverslip were calculated by averaging the halo intensity of the two least intensely (0%) and two most intensely stained cell profiles (100%). Neurons were considered IB4 positive (IB4+) if their relative intensities exceeded 20%.

2.6. Electrophysiology. Electrophysiological recordings were performed using a standard whole-cell technique [27]. Electrodes were pulled from borosilicate glass microcapillaries with a filament (Sutter Instrument, Novato, CA) and had a resistance of 3 to 4 MΩ when filled with an internal solution containing (in mM) 146 CsCl, 2 MgATP, 2 $MgCl_2$, 0.5 GTP-Na, 1 EGTA, 5 2Na-phosphocreatine, and 10 HEPES, adjusted to pH 7.3 with CsOH. The external solution for calcium currents recording contained (in mM) 2 $CaCl_2$, 2 $MgCl_2$, 158 tetraethylammonium- (TEA-) Cl, 10 glucose, and 10 HEPES adjusted to pH 7.4 with TEA-OH. Electrophysiological recordings were performed using an EPC-10/2 amplifier controlled by PatchMaster software (HEKA, Freiburg, Germany). FitMaster software (HEKA, Freiburg, Germany) was used for offline data analysis. Currents were low-pass filtered at 2–5 kHz. A T-type calcium current was evoked by step pulse to −45 mV for 500 ms after preconditioning at potential of −95 mV for 3 s. Bath application of Tyrode's solution supplemented with capsaicin (2 μM) was used to test capsaicin sensitivity at the end of experimental procedure. Multiple independently controlled glass syringes served as reservoirs for a gravity-driven local perfusion system. All drugs were prepared as stock solutions: capsaicin (10 mM) in DMSO, Ni^{2+} (100 mM), and mibefradil (5 mM) in H_2O. Drugs were freshly diluted to the appropriate concentrations at the time of experiments. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted.

2.7. Analysis. Statistical comparisons were performed using unpaired Student's t-test, one-way ANOVA, and Fisher's exact test. All quantitative data are expressed as means of multiple experiments ± SEM. The amplitude of T-type current was measured as a difference between the current peak value and the current value at the end of a depolarizing command pulse in order to avoid a residual HVA current. Activation and inactivation kinetics were estimated for each recorded T-type current as time constants of two-exponential fit from 10% of amplitude at rising part to the end of an evoking step. Voltage dependencies of activation and steady-state inactivation were

described in a standard way [27] using single Boltzmann distributions of the following forms:

$$\text{activation } \frac{G(V)}{G_{\max}} = \frac{1}{1+\exp\left(-\left(V-V_{1/2}\right)/k\right)},$$
$$\text{inactivation } \frac{I(V)}{I_{\max}} = \frac{1}{1+\exp\left(\left(V-V_{1/2}\right)/k\right)}, \quad (1)$$

where conductance ($G(V)$) was defined as PCD/($V - E_r$) (PCD is a *peak current density* defined as I_{peak}/C_m and ($V-E_r$) is *an electrodriving force* for a membrane potential (V) and a reversal potential (E_r) obtained from interpolation of $I(V)$ dependence); $G_{\max}$ is the maximal conductance and $I_{\max}$ is the maximal peak current amplitude; $V_{1/2}$ is a voltage at which half of the current is activated or inactivated, and k represents the slope factor of voltage dependence. The fitted values for $V_{1/2}$ and k are reported with 95% linear confidence limits.

3. Results

3.1. Different Types of PDN Induced by STZ Diabetes. Three days after diabetic induction by an injection of STZ, most (70%) rats developed strong hyperglycemia (mean glucose concentration 29 ± 2 mM) and were considered diabetic. As reported previously [27], within 6-7 weeks after injection of STZ, hyper-, hypo-, and normalgesic types of PDN were present in the population of STZ diabetic rats that was determined based on changes in a paw withdrawal latency (PWL). The animals were considered as thermally hyperalgesic if their PWL was less than 8.9 s, hypoalgesic if it was longer than 15.5 s, and normalgesic in any other cases (unchanged response, PWL within 8.9 ÷ 15.5 s) [27]. Animals with thermal hyperalgesia ($n = 12$), normalgesia ($n = 8$), and hypoalgesia ($n = 9$) were selected from the population of rats with 6-7 weeks of STZ-induced diabetes. The averaged PWLs for hyper-, hypo-, and normalgesic groups were 7.7 ± 0.3 s, 17.4 ± 0.5 s, and 12.4 ± 0.7 s, correspondingly, while the averaged PWL in control was 12.0 ± 0.7 s ($n = 10$). In agreement with the previous study [27], the blood glucose level of diabetic animals was significantly different from that of the control rats. However, no significant differences in the blood glucose level and body weight were observed between experimental rats of different diabetic groups (data not shown).

Thus, simultaneous presence of hyper-, norm-, and hypoalgesic animals was confirmed within the population of rats with 6-7 weeks of STZ-induced diabetes. These three animal groups together with control animals were further used to analyze whether there were thermal C-fiber nociceptive neurons specifically expressing fast or slow T-currents and whether there are some changes in expression of these currents associated with the different types of PDN induced by type 1 diabetes.

3.2. NTCN Neurons Expressing Fast or Slow T-Type Ca^{2+} Currents. T-current was recorded in nonpeptidergic thermal C-type nociceptors (NTCN) [32] that are strongly involved in thermal pain sensitivity [33] and neuropathic pain [34, 35]. To separate these neurons from other types of small-size DRG neurons, a population of freshly isolated cells was stained with isolectin B4 (IB4) [37] for *in vitro* labeling of nonpeptidergic neurons [32]. IB4-positive small-size neurons (Figure 1(a) (A)) were held in a voltage clamp mode at −60 mV and challenged with TRPV1 channels agonist, capsaicin. Neurons which responded to capsaicin application (2 μM, 15 s) with an inward current (Figure 1(a) (B)) were considered as NTCN neurons.

T-current was recorded in these neurons using a voltage step to −45 mV after preconditioning at −95 mV. Its kinetics of inactivation (τ_{in}) was calculated as a time constant of a single-exponential fit of decay. A strong variability of time constant values was observed within the population of neurons under study allowing suggesting that fast and slow T-current could be specifically expressed in different neurons (Figure 1(b)). A pooled distribution of τ_{in} values was built for T-currents recorded in neurons taken from control ($n = 43$), hyper- ($n = 15$), hypo- ($n = 14$), and normalgesic ($n = 14$) animals (Figure 1(c)) to statistically test whether different T-currents were expressed in the NTCN neurons. The distribution of τ_{in} values was strongly right-skewed. A Shapiro-Wilk normality test also indicated that it was not Gaussian ($P < 2 \cdot 10^{-6}$). At the same time, the distribution was reasonably fitted by two Gaussians (Figure 1(c)) suggesting the division the T-current into fast ($\tau_{\text{in}} < 50$ ms) and slow ($\tau_{\text{in}} > 50$ ms) subtypes. The average τ_{in} of the slow T-current was almost three times larger than the one of the fast T-current (Figure 1(d); Table 1). Surprisingly, in the control group a peak current density (PCD) of the slow T-current was almost twice as large as the fast one (Figure 1(e); Table 1; $P < 0.001$). Since slower decay kinetics was observed in neurons having larger PCD, this slower decay could be due to a voltage clamp problem rather than due to a difference in T-type channel gating. If this were the case, positive correlation between the T-current amplitude and kinetics of inactivation should be observed within neuronal populations expressing both fast and slow T-currents. However, no significant correlation was found between PCD and τ_{in} of fast and slow T-currents in the control group (Figure 1(f)), suggesting that the difference in τ_{in} between fast and slow T-currents was not an artifact of poor voltage clamp. Moreover, there was no significant difference in macroscopic activation kinetics between fast and slow T-currents (Table 1) which also confirmed that it is a difference in T-type channel gating that underlies fast and slow kinetics of the T-current. Thus, two groups of neurons were identified among the whole population of NTCN neurons based on their difference in T-current inactivation. It is also interesting to note that capacitance of cells expressing the slow T-current was significantly smaller (about 30%) than those expressing the fast T-current (Table 1; $P < 0.01$). This also suggests that two different neuronal groups express different T-currents.

3.3. $Ca_v3.2$ (α1H) Isoform of T-Type Channels Differently Contributes to Fast and Slow T-Type Ca^{2+} Currents Expressed in NTCN Neurons. $Ca_v3.2$ isoform of T-type channels is the most abundantly expressed in DRG neurons [26] and

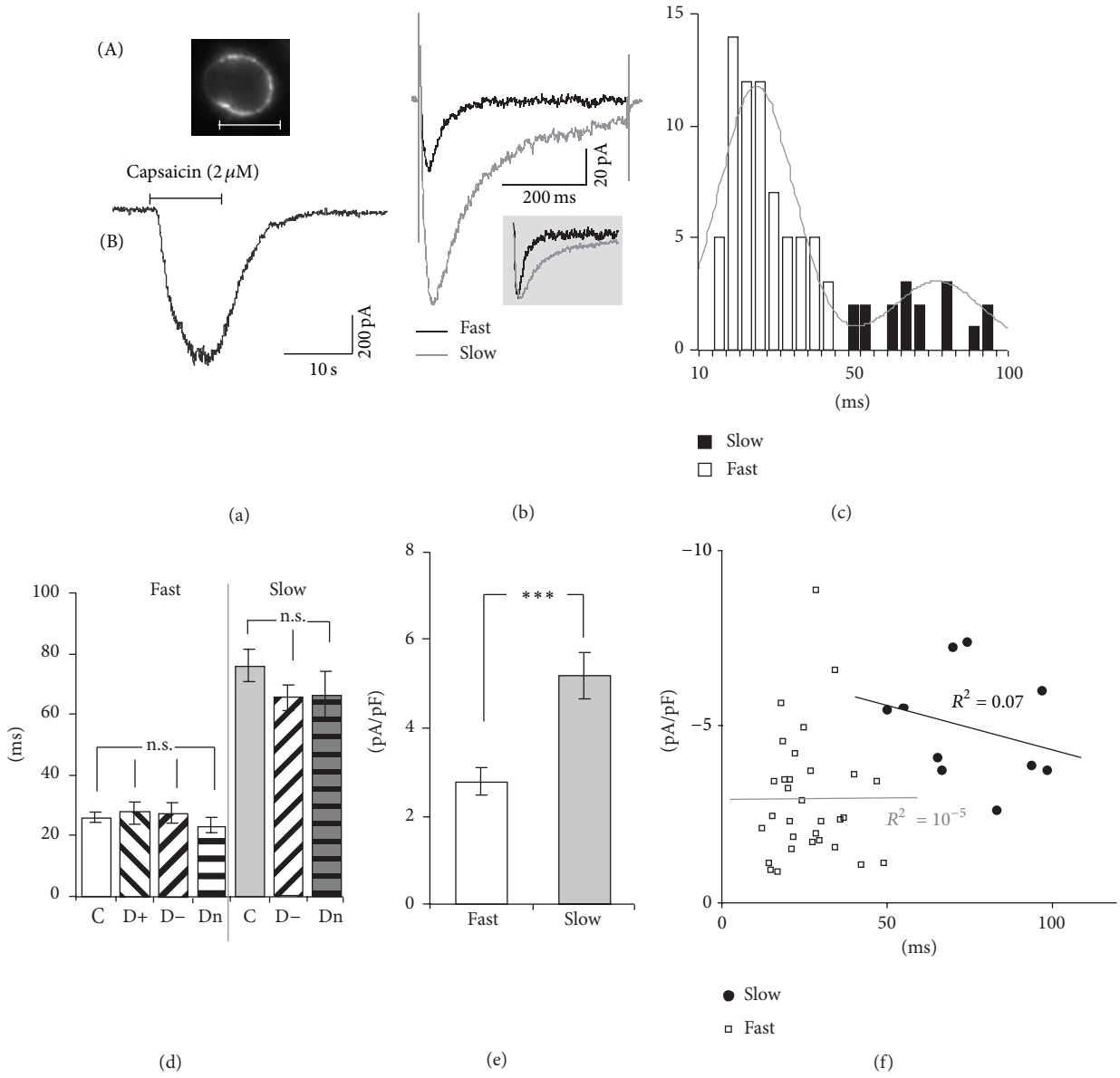


Figure 1: NTCN neurons express both fast and slow T-currents. (a) Identification of NTCN neurons. (A) A typical fluorescent image of an IB4-positive small-size DRG neuron. Note the intensive fluorescent ring associated with the neuronal plasma membrane. Scale bar, 20 μm. (B) A typical trace of transmembrane current induced by application of capsaicin (2 μM) in IB4-positive small-sized DRG neuron. IB4-positive capsaicin-sensitive small size DRG neurons were further considered as nonpeptidergic thermal C-type nociceptive (NTCN) neurons. (b) Representative current traces illustrate expression of T-currents with fast and slow kinetics of inactivation in different NTCN neurons. Currents were elicited using a 0.5 s voltage step to −45 mV after preconditioning at −95 mV for 3 s. A grey inset shows the same currents normalized by amplitude to underline a difference in kinetics of current inactivation. (c) A histogram demonstrates a pooled distribution of inactivation time constants of T-currents recorded from 85 neurons of control and PDN groups. The time constants were calculated from a single-exponential fit of current decay. A smooth curve is a fit of the distribution by a sum of two Gaussians. According to this fit T-currents were divided into fast (τ_{in} < 50 ms; white bars) and slow (τ_{in} > 50 ms; black bars) subtypes. (d) Kinetics of inactivation of fast and slow T-currents in control and PDN groups. Each column is the mean and SEM from the number of neurons specified in Figure 2(a). No significant difference compared to control was revealed under PDN conditions in kinetics of inactivation for both fast and slow T-currents. (e) Peak current density (PCD) of fast and slow T-currents under the control conditions. The columns are the mean and SEM calculated from 31 fast and 12 slow T-currents. $^{***}P$ < 0.001. (f) PCD plotted *versus* inactivation time constant for fast and slow T-currents recorded under the control conditions. No significant correlations were found for both current types indicating that the difference in inactivation between fast and slow T-currents was not due to voltage clamp problems. Lines were liner fits of the dependencies; R^2 as a measure of correlation is shown in the plot.

Table 1: Parameters of fast and slow T-currents in NTCN neurons.

Control		C, pF		T-current parameters at −45 mV					
				Peak current density, pA/pF		Time constant of activation, ms		Time constant of inactivation, ms	
		Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
		18.8 ± 1.0	12.8 ± 1.5	2.8 ± 0.3	5.2 ± 0.5	6.6 ± 0.6	7.2 ± 0.8	26 ± 2	76 ± 5
Diabetes	Hyperalgesia	15.5 ± 1.4		4.4 ± 0.4		5.0 ± 0.9		27 ± 4	
	Hypoalgesia	17.9 ± 1.0	17.7 ± 1.0	2.6 ± 0.4	2.0 ± 0.6	7.7 ± 1.6	6 ± 1	27 ± 3	66 ± 4
	Normalgesia	2.6 ± 2.2	13.3 ± 1.0	2.5 ± 0.5	1.9 ± 0.5	6.1 ± 0.7	3.2 ± 0.3	23 ± 2	66 ± 8

mediates the most part of T-current in NTCN neurons [27]. However, it has been reported recently that $Ca_v3.3$ can also perceptibly contribute to the T-current in a subpopulation of small DRG neurons resulting in slower inactivation of the T-current these neurons express [28]. In order to examine a functional contribution of $Ca_v3.2$ channels to the fast and slow T-currents in NTCN neurons of naive animals we used low micromolar concentrations of Ni^{2+} known to be a specific blocker of $Ca_v3.2$ isoform with no significant effect on $Ca_v3.1$ and $Ca_v3.3$ T-type channel subtypes [13, 38]. A specific T-type channel blocker mibefradil [13], which is not subunit specific, was additionally used to confirm that an electrophysiologically isolated LVA current is mediated by T-type channels.

As expected, Ni^{2+} at low micromolar concentration (50 μM) significantly and reversibly blocked both fast and slow T-currents ($P < 0.001$, Figure 2(a)). At the same time the fast T-current was significantly more sensitive to Ni^{2+} than the slow one (18 ± 3% ($n = 6$) compared to 28 ± 1% ($n = 3$) of initial current persisted for the fast and slow T-currents, resp.) ($P < 0.02$, Figure 2(c)). This finding points to a significantly larger contribution of $Ca_v3.2$ isoform to the fast than to the slow T-current. There was no significant difference in kinetics of inactivation between Ni^{2+}-sensitive (27 ± 2 ms) and Ni^{2+}-insensitive (30 ± 2 ms) components of fast T-current as well as the fast T-current itself (26 ± 2 ms; ANOVA, $P = 0.6$; insets in Figures 2(a) and 2(b)). It is interesting to note that kinetics of inactivation of Ni^{2+}-sensitive component of slow T-current (32 ± 2 ms) was not significantly different from those of Ni^{2+}-sensitive component of fast T-current and the fast T-current itself (ANOVA, $P > 0.3$; Figures 2(b) and 2(e)), suggesting that this component is also mediated by $Ca_v3.2$ isoform. In contrast, kinetics of inactivation of Ni^{2+}-insensitive component of slow T-current (Figure 2(a), right inset) was 1.78 ± 0.03-fold slower than one of the total slow T-current. This ratio was significantly different compared to the respective ratio calculated for the fast T-current (1.15 ± 0.10; $P < 0.01$; Figure 2(d)). In addition, for the slow T-current, Ni^{2+}-insensitive component was substantially and significantly slower (2.69 ± 0.12 times) than the Ni^{2+}-sensitive one ($P < 0.001$; Figure 2(b), right inset), suggesting that these components are mediated by different T-channel isoforms. Altogether these results confirm our earlier suggestion about a significantly larger contribution of $Ca_v3.2$ isoform to the fast than to the slow T-current. Finally, mibefradil (10 μM) blocked 93 ± 3% and 93 ± 1% of fast and slow T-currents, respectively. The effect was significant ($P < 0.001$) with no significant difference between the fast and slow T-currents ($P = 0.95$), thus providing additional pharmacological confirmation that recorded fast and slow currents were mediated by T-channels.

Taken together these findings demonstrate significantly different pharmacological properties of T-channels mediating the fast and slow T-currents in NTCN neurons. While the major part of both the fast and slow T-currents seems to be mediated by $Ca_v3.2$ T-type channel isoform, a considerable contribution of the other T-channel isoforms was established for the case of slow T-current.

3.4. PDN-Specific Functional Expression of Fast and Slow T-Currents in NTCN Neurons. In the next phase of our research, we determined whether the NTCN neurons with the slow T-current were present under diabetic conditions and whether their percentage was differentially changed in animals with different types of PDN. We demonstrated that NTCN neurons of hypo- and normalgesic animals expressed either slow or fast T-currents, as was observed in control conditions (Figure 3(a)). The slow T-current was found in about 30% of the neurons in each of these diabetic groups with no significant differences in their fraction between both these groups and control ($P > 0.05$, Fisher's exact test). This suggests that the distribution of slow and fast T-currents among NTCN neurons was not affected in hypo- and normalgesic PDN. In contrast, the NTCN neurons of hyperalgesic rats only expressed the fast T-current (15 of 15 tested cells; Figure 3(a)). The percentage of NTCN neurons expressing the slow T-current differed significantly between hyperalgesic and control groups ($P < 0.05$, Fisher's exact test), suggesting specific abolishment of the slow T-current or elimination of the respective NTCN neurons under hyperalgesic PDN. Under diabetic conditions we did not observe a difference in capacitance between NTCN neurons expressing the slow and fast T-currents in the hypoalgesic group. However, a significant difference in capacitance was preserved in normalgesic group (Table 1; $P < 0.01$) as was initially established for the neurons of the control group.

In addition, we analyzed possible effects of differential diabetes development under different PDN on functional expression of fast and slow T-currents. We calculated the PCD of fast and slow T-currents and compared these

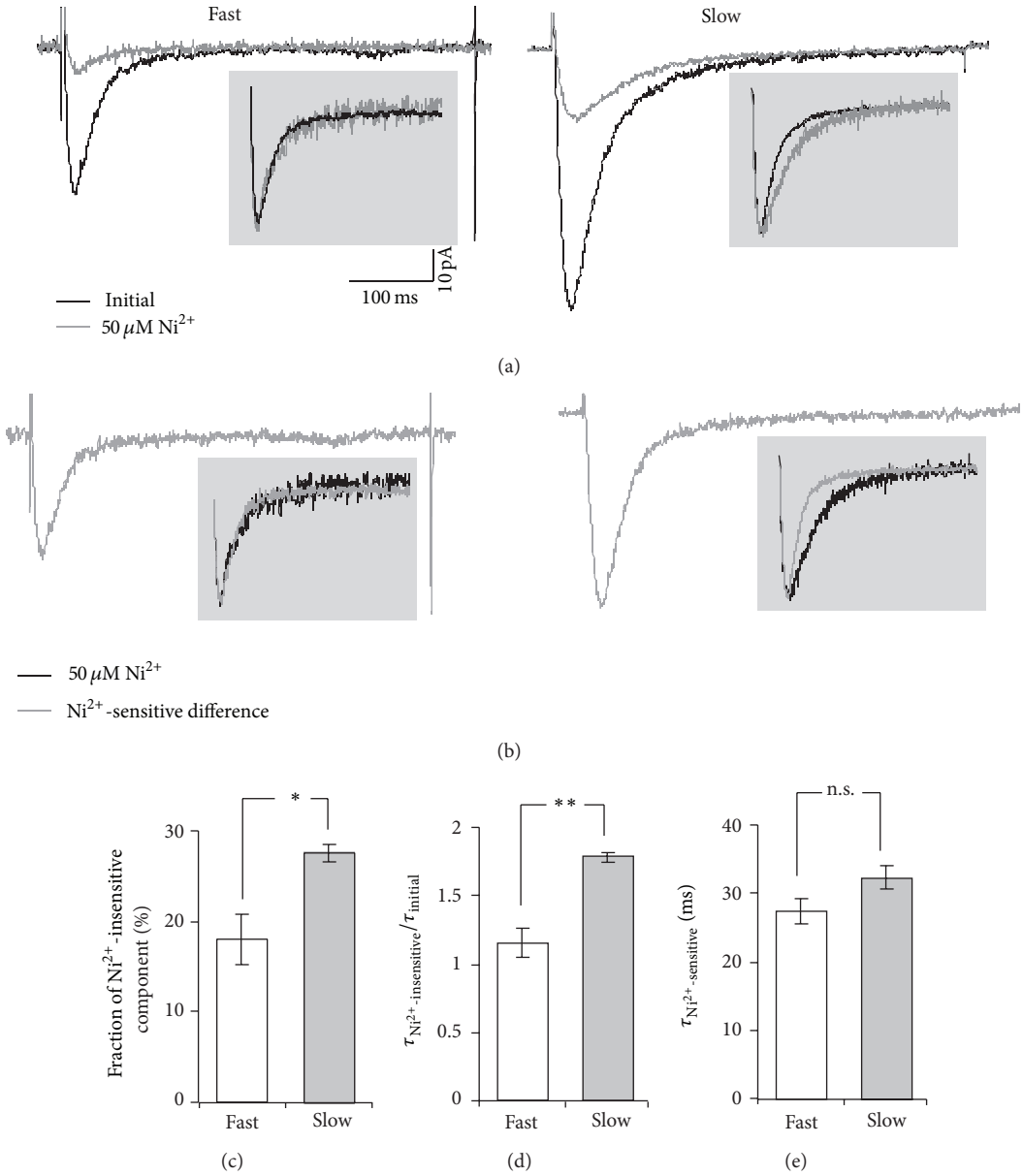


Figure 2: Fast and slow T-currents expressed by NTCN neurons reveal different sensitivity to low Ni^{2+} concentration. (a) Representative current traces illustrate effect of Ni^{2+} application to NTCN neurons of naive rats expressing fast (left) and slow (right) T-currents. Initial (total) T-current traces are shown in black while grey traces represent a residual Ni^{2+}-insensitive component of T-current persisted during Ni^{2+} application. Note the considerably larger blocking effect of Ni^{2+} application on the fast compared to slow T-currents. Insets show the total and Ni^{2+}-insensitive currents normalized by their amplitudes in order to directly compare their inactivation kinetics further shown in (c). Note the slower inactivation of Ni^{2+}-insensitive component compared to the total current for the case of slow T-current. Scale bars shown in (a) are applicable to all current traces in (a) and (b). (b) Representative traces for a Ni^{2+}-sensitive component of fast (left) and slow (right) T-currents were obtained by digital subtraction of the Ni^{2+}-insensitive component from the total T-current for traces shown in (a). Insets demonstrate normalized Ni^{2+}-sensitive (gray) and Ni^{2+}-insensitive (black) components. Note the absence of visible difference in kinetics of inactivation between these components of the fast T-current and a substantially slower Ni^{2+}-insensitive component as compared to the Ni^{2+}-sensitive one for the case of slow T-current. (c) Fractions of Ni^{2+}-insensitive component in the fast and slow T-currents were significantly different. *-$P < 0.05$. (d) A ratio of inactivation kinetics of Ni^{2+}-insensitive component and the total T-current for NTCN neurons expressing the fast and slow T-currents. There were no significant changes observed in the case of fast T-current ($P > 0.4$), while the inactivation kinetics of Ni^{2+}-insensitive component of slow T-current was significantly slower compared to the inactivation kinetics of the total current. **-$P < 0.01$. (e) Inactivation kinetics of Ni^{2+}-sensitive components of fast and slow T-currents. n.s.: no significant difference was revealed between the inactivation kinetics of Ni^{2+}-sensitive components of the fast and slow T-currents ($P > 0.3$). Each column in (c), (d), and (e) is the mean and SEM from 6 fast and 3 slow T-currents.

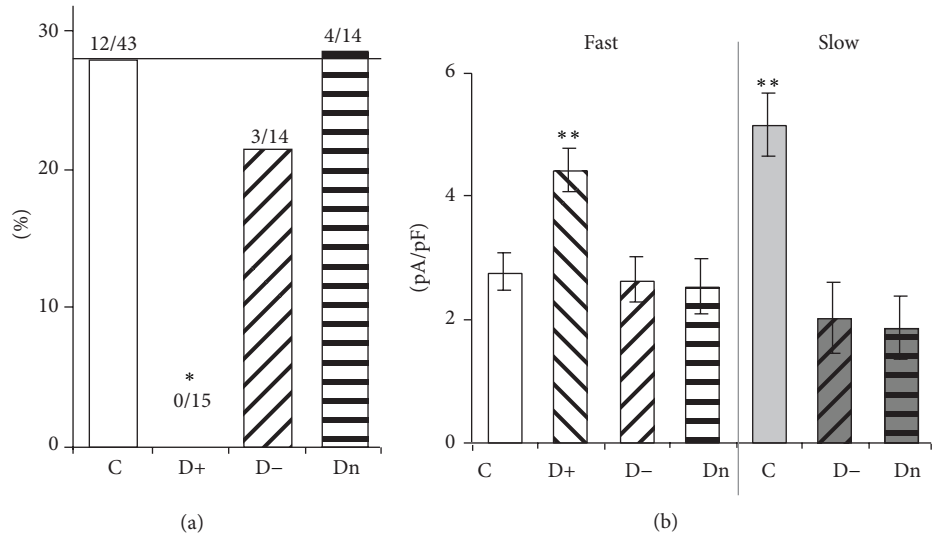


Figure 3: Functional expression of fast and slow T-currents in NTCN neurons under different PDN conditions. (a) Percentage of NTCN neurons revealing the slow T-current in control (C), hyper- (D+), hypo- (D−), and normalgesic (Dn) groups. The slow T-current was not observed under hyperalgesic conditions, $^{*}P < 0.05$ (Fisher's exact test). The numbers above the columns indicate the number of NTCN neurons expressing the slow T-current of the total number of tested neurons in the respective group. (b) PCD of fast and slow T-currents under the control and PDN conditions. It is interesting to note that the fast T-current was upregulated in hyperalgesic conditions while the slow T-current was strongly downregulated in norm- and hypoalgesia. Each column was the mean and SEM from number of neurons specified in (a). $^{**}P < 0.01$ (ANOVA). n.s.: not significant (ANOVA).

pairwise between control, hyper-, hypo-, and normalgesic animal groups. It was found that PCD of fast T-current was substantially and significantly increased only in the hyperalgesic group (by 60 ± 20%) compared with the control group, while no significant changes were observed in hypo- and normalgesic diabetic groups (Figure 3(b); Table 1). At the same time, the slow T-current, absent in the NTCN neurons under hyperalgesia, was significantly decreased in the hypo- (by 61 ± 12%) and normalgesic (by 64 ± 10%) groups compared with the control group (Figure 3(b); Table 1). It is interesting to note that the PCD of slow and fast T-currents, which were significantly different in the control group, did not differ in the neurons of hypo- and normalgesic diabetic groups (Figure 3(b); Table 1) indicating an increased relative contribution of the fast T-current in neuronal Ca^{2+} signaling.

3.5. PDN Alters Biophysical Properties of Fast and Slow T-Currents in NTCN Neurons. Changes in biophysical properties of T-channels may substantially influence the neuronal excitability [39]. Moreover, changes in voltage-dependent activation and steady-state inactivation (SSI) of T-channels have been recently reported for different types of PDN [27]. Therefore, the biophysical properties of fast and slow T-currents were also examined in NTCN neurons under different types of PDN (Table 2). We found that the voltage-dependent activation and SSI of fast and slow T-currents were not significantly different in control conditions (ANOVA, $P > 0.2$) (Table 2). We also determined that macroscopic activation and inactivation kinetics of the fast T-current were not significantly different between NTCN neurons of control, hyper-, hypo-, and normalgesic groups (Table 2; ANOVA, $P > 0.3$). Analogous results were obtained for the slow T-current recorded in control, hypo-, and normalgesic groups of rats (Table 2; ANOVA, $P > 0.05$). Our results suggest that activation and inactivation kinetics of fast and slow T-currents were not significantly affected under different types of PDN compared to control. No significant differences were also found in the half-activation potentials and slope factors of T-current activation and inactivation between any of the groups (control and all PDN) and in the current type (fast or slow) T-currents (Table 2; ANOVA, $P > 0.5$). At the same time, the voltage-dependence of SSI revealed a significant depolarizing shift (about 8 mV) in the half-inactivation potential of the fast T-current under hyperalgesia and of the slow T-current under hypo- and normalgesia (Figure 4) (ANOVA, $P < 0.02$), compared with the control group, Thus, activation properties of T-type channels seemed to be unaffected under PDN conditions. In contrast, SSI of slow and fast T-type currents was found to be specifically shifted in a similar way in NTCN neurons expressing slow T-current under hypo- and normalgesic PDN and fast T-current under hyperalgesic PDN.

Accordingly, we have found that two different subtypes of T-type currents distinguished by their inactivation were specifically altered under hyper-, hypo-, and normalgesic STZ-diabetic neuropathy with a prominent difference between patterns of changes observed in hyperalgesia *versus* hypo- and normalgesia.

4. Discussion

Recently it has been demonstrated that differences in thermal pain sensitivity between hyperalgesic, hypoalgesic, and

Table 2: Parameters of T-current activation and steady-state inactivation.

Control		Steady-state inactivation				Activation			
		$V_{1/2}$, mV		k, mV		$V_{1/2}$, mV		k, mV	
		Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
		−90 ± 2	−88 ± 4	5.9 ± 0.6	5.7 ± 0.7	−49.3 ± 1.3	−46 ± 2	5.1 ± 0.7	5.7 ± 1.2
Diabetes	Hyperalgesia	−81.3 ± 1.7		6.0 ± 0.2		−48.9 ± 1.6		5.2 ± 0.3	
	Hypoalgesia	−96 ± 4	−82 ± 2	4.3 ± 1.6	5.3 ± 0.5	−50 ± 4	−49.9 ± 1.7	7 ± 3	4.1 ± 0.8
	Normalgesia	−85 ± 2	−80.1 ± 1.0	5.6 ± 0.4	6.4 ± 0.2	−51.7 ± 1.4	−49 ± 2	6.3 ± 0.9	5.9 ± 0.8

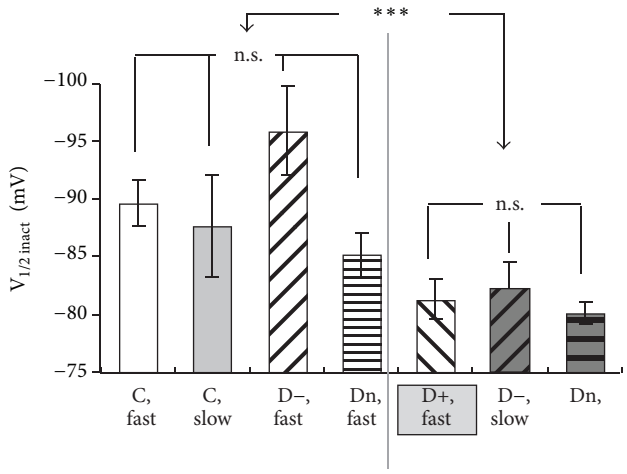


Figure 4: PDN-specific changes in steady-state inactivation of T-currents in NTCN neurons. Each column demonstrates the mean and SEM of half-inactivation potential of steady-state inactivation (SSI) calculated for 13 "fast" and 7 "slow" neurons of control group (C), 7 neurons of hyperalgesic group (D+), 5 "fast" and 3 "slow" neurons of hypoalgesic group (D−), and 8 "fast" and 4 "slow" neurons of normalgesic group (Dn). The results demonstrate that a depolarization shift in SSI was observed for the fast T-current in a case of hyperalgesia and for the slow T-current in norm- and hypoalgesia. ANOVA between all columns produced $P < 0.02$. $^{***}P < 0.001$ (t-test for merged "C, fast," "C, slow," and "D−, fast," "Dn, fast" *versus* merged "D+, fast," "D−, slow," and "Dn, slow"). n.s.: not significant (ANOVA).

normalgesic diabetic rats are likely due to differential changes in the functioning of TRPV1 and T-channels within a pool of NTCN neurons [27], a subclass of nonpeptidergic primary nociceptors terminating in lamina II and playing an important role in neuropathic pain [32]. In this study, functioning of T-channels in NTCN neurons was further investigated using the same experimental model of STZ-induced diabetic neuropathy. To our knowledge, this is the first study demonstrating that T-channels underlying fast and slow LVA Ca^{2+} currents are heterogeneously expressed in NTCN neurons and are specifically modulated under thermal hyper-, hypo-, and normalgesia accompanying STZ diabetes. Our results provide better understanding of potential molecular mechanisms involved in the expression of different types of PDN.

As in humans, development of PDN in rats is accompanied with various alterations in pain sensation (hyper- and, hypoalgesia, and allodynia) or leaves pain sensation unchanged (normalgesia) [2–5]. These alterations can be considered as a manifestation of different types of PDN [27]. In the current study, STZ-diabetic rats revealing different modalities of thermal nociception and simultaneously having the same age and terms of diabetes development were used as a model for the investigation of PDN-type-specific remodeling of T-type Ca^{2+} channels involved in nociception. No difference was observed in blood glucose levels and weight between rats with different modalities of thermal nociception, which suggests that the PDN-type-specific remodeling of T-type Ca^{2+} channels found in this work is unlikely due to a different metabolic state of the experimental animals and is probably directly related to a particular type of PDN.

4.1. NTCN Neurons Expressing Fast and Slow T-Currents. Small DRG neurons differ in the biophysical properties of expressed T-currents. About 65% of small DRG neurons express a fast inactivating T-current having biophysical and pharmacological properties resembling those of a current mediated by the T-channels of $Ca_v3.2$ subtype [28, 30]. At the same time the other 35% of the neurons express a slowly inactivating T-current consisting of two pharmacologically separable components. This has been attributed to a different Ca_v3 subunits composition expressed by these neurons [28]. Therefore, a heterogeneous population of small DRG neurons can be divided into two classes [28] that can be in particular distinguished by a rate of T-current inactivation. Differences in sensitivity to capsaicin and mechanical stimulation between these two classes of small DRG neurons [28] allowed assuming that these neurons might also be functionally different. According to our results, NTCN neurons may be also divided into two classes characterized by the expression of either a fast or slow T-current, correspondingly. The fraction of "slow" neurons (~30%) observed in our experiments is also close to the reported one for the whole population of small DRG neurons [28]. The "slow" neurons were slightly (~30%) but significantly smaller and exhibited significantly lager (~2-fold) T-currents than "fast" ones, which is also in good agreement with previous findings [28].

4.2. T-Type Ca^{2+} Channels Mediating Fast and Slow T-Currents. A difference in kinetics of inactivation between the fast and slow T-currents observed in this research could arise from differential expression of various isoforms of T-type channels. Indeed, two isoforms of T-type channels, $Ca_v3.2$

and $Ca_v3.3$, have found to be expressed in DRG neurons [26, 40]. $Ca_v3.2$ is the most abundant isoform [26] both within the soma and peripheral axons of small and medium DRG neurons [41] and it mainly underlies the T-type current in small [20] (including NTCN [27]) and medium [25] DRG neurons, classically considered as nociceptive. Coste et al. have recently suggested that a proportion of small DRG neurons functionally express the $Ca_v3.3$ isoform [28]. The T-currents mediated by the $Ca_v3.2$ channels are known to have fast inactivation ($\tau_{inact} \sim 20$ ms) while the $Ca_v3.3$-mediated current exhibits substantially slower kinetics of inactivation ($\tau_{inact} \sim 70$ ms) [13, 38]. These data are in a good agreement with our results in respect of inactivation time constants of fast and slow T-currents and suggest that the fast T-current might be mediated by $Ca_v3.2$ channels, whereas $Ca_v3.3$ channels may contribute to the slow T-current.

Previous findings indicate that blockers of $Ca_v3.2$ channels substantially suppress T-currents in the whole population of small DRG neurons, including the NTCN ones [20, 27]. In this study Ni^{2+} at a low micromolar concentration also significantly blocked both fast and slow T-currents (Figure 2(a)), suggesting high contribution of $Ca_v3.2$ to both of them. At the same time the fast T-current was found to be significantly more sensitive to Ni^{2+} than the slow one (Figure 2(c)). Together with the slower kinetics of inactivation of Ni^{2+}-insensitive compared to Ni^{2+}-sensitive component of the slow T-current (Figure 2(b), right inset) this finding additionally confirms a contribution of $Ca_v3.3$ channel isoform to the slow T-current.

It was found in this study that 82% of fast T-current was blocked by 50 μM of Ni^{2+}. This value is close to 81% calculated for Ni^{2+}-induced block of $Ca_v3.2$-mediated current based upon $IC_{50} = 10.3\,\mu$M and $n = 0.9$ reported in [25]. It allows us to assume that the fast T-current is solely mediated by $Ca_v3.2$ channel isoform (including 18% of the residual current observed in the presence of 50 μM of Ni^{2+}). This assumption is strongly supported by the fact that the kinetics of inactivation of the fast T-current itself as well as its Ni^{2+}-sensitive and Ni^{2+}-insensitive components seems to be the same (insets in Figures 2(a) and 2(b)). If the same extent of Ni^{2+}-induced block of $Ca_v3.2$-mediated current is present in the slow T-current then one can estimate from Figure 2(c) that $Ca_v3.3$ channel isoform contributes about 12% to an amplitude of the slow T-current. This value is close to 15% observed by Coste et al. for $Ca_v3.3$-mediated current in a proportion of small DRG neurons [28]. It is interesting to note that, despite a relatively low contribution to the amplitude, $Ca_v3.3$ isoform accounts for about 30% of charge transferred by the slow current since kinetics of inactivation of $Ca_v3.3$-mediated current is at least 2.69 times slower (Figure 2(b)). Thus, it seems very likely that coexpression of $Ca_v3.2$ and $Ca_v3.3$ isoforms of T-channels underlies the slow T-current in a subpopulation of NTCN neurons. At the same time, according to our and others' results [28], the NTCN neurons may hardly express the $Ca_v3.3$ channel isoform alone.

A difference in kinetics of inactivation between the fast and slow T-currents could be also explained by expression of $Ca_v3.2$ splice variants having slower inactivation kinetics. It might be a promising hypothesis since a high contribution of $Ca_v3.2$ to both fast and slow T-currents is found in this study (Figures 2(a) and 2(c)). However, the expected difference in inactivation kinetics between the $Ca_v3.2$ splice variants [42] is less than found between fast and slow T-currents in this work and, therefore, could not completely account for the experimental observations. At the same time, expression of splice variants may partially contribute to the variations of kinetics observed within the fast and slow types of T-currents.

Thus, expression of various T-type channel isoforms possibly in concert with their different splice variants may account for the difference in inactivation kinetics between the fast and slow T-currents found in this work. Most probably, a population of NTCN neurons consists of two different classes. One of them solely expresses $Ca_v3.2$ channels mediating the fast T-current while the other one expresses a mixture of $Ca_v3.2$ and $Ca_v3.3$ isoforms thus far producing the slow T-current.

4.3. Fast and Slow T-Currents in NTCN Neurons under Different Types of PDN. Differences in functioning of T-channels in NTCN neurons have been recently proposed as an important factor resulting in different modalities of thermal nociception under STZ-induced diabetic neuropathy [27]. The importance of $Ca_v3.2$ channels in peripheral nociceptive signaling was established previously, including a key role of their upregulation in hyperalgesia under STZ diabetes and chronic constrictive injury [16–25, 43–48]. Although changes in $Ca_v3.3$ functional expression have not been documented under PDN, upregulation of $Ca_v3.3$ was recently implicated in the sensitization of small DRG neurons, which possibly underlies hyperalgesia in the model of spinal nerve injury [40].

Absence of "slow" NTCN neurons, most probably expressing both $Ca_v3.2$ and $Ca_v3.3$ channels, under hyperalgesic PDN seems to be the very interesting finding of this work. It is hardly related to diabetes-induced death of these neurons since, to the best of our knowledge, no substantial damage of small DRG neurons has been reported at 6-7 weeks of STZ-induced diabetes. At the same time, diabetes-induced upregulation of $Ca_v3.2$ channels simultaneously with downregulation of $Ca_v3.3$ channels may substantially accelerate inactivation of the total T-current. In this case, NTCN neurons still expressing a mixed set of Ca_v3 channels could be classified as "fast." This seems to be a quite realistic scenario since significant upregulation of the $Ca_v3.2$ channels is a common feature of hyperalgesic PDN [20, 25, 27, 45]. Our findings of a significant increase of T-current in "fast" (presumably $Ca_v3.2$ expressing) NTCN neurons under hyperalgesic conditions and a significant decrease of T-current in "slow" NTCN neurons under norm- and hypoalgesic conditions also support this explanation. $Ca_v3.3$ downregulation could be, in general, a common feature of all types of PDN observed in this study. This is supported by our findings of the absence of slow T-current in hyperalgesic animals and its significant decrease in hypo- and normalgesic rats. It is interesting to note that unchanged kinetics of slow T-current inactivation together with its decrease in hypo-

and normalgesic rats suggests downregulation of both $Ca_v3.3$ and $Ca_v3.2$ channels in the neurons expressing the slow T-current without changes in a ratio between $Ca_v3.2$ and $Ca_v3.3$ channels. At the same time, no significant changes in the T-current were observed in "fast" NTCN neurons of hypo- and normalgesic rats, suggesting different sensitivity of T-channel modulation in "fast" and "slow" NTCN neurons during the progress of STZ diabetes.

Another interesting finding of the current study is a depolarizing shift in voltage dependence of inactivation under STZ-induced diabetes. A similar shift in a half-inactivation potential was also reported in our previous study [27]. The novel finding of the current study is that the shift was observed only in the "fast" NTCN neurons of hyperalgesic rats and "slow" NTCN neurons of hypo- and normalgesic rats. The shift may contribute to an increase in neuronal excitability [39] of NTCN neurons possibly underlying thermal hyperalgesia under STZ diabetes [27].

Functional expression of different Ca_v3 channels may influence Ca^{2+} signaling associated with different neuronal activities due to specific biophysical properties of these channels. $Ca_v3.2$ channels were shown to be underlying after depolarization potentials and participating in rebound discharge in medium [25] and T-rich [49] DRG neurons. However, relatively fast inactivation and slow recovery from inactivation limit the ability of $Ca_v3.2$ channels to respond to high frequency stimulation (>20 Hz). The role of $Ca_v3.3$ channels was not profoundly studied in peripheral sensory neurons. At the same time it was shown that slowly inactivating $Ca_v3.3$ channels can still contribute to Ca^{2+} entry during high frequency bursts (100 Hz) and slow, prolonged, or repetitive stimulations [50, 51] when $Ca_v3.2$ channels already became inactive. It seems reasonable to assume that, in "slow" NTCN neurons, $Ca_v3.3$ channels play similar role, being a sensor of high frequency bursting and slow, prolonged, or repetitive nociceptive input, thus mediating Ca^{2+} entry in response to such activities. Taken together, these considerations allow us to suggest the following functional consequences of observed changes in T-channels functioning. An almost 2-fold increase in T-channel PCD together with the shift of its half-inactivation potential in the NTCN neurons of hyperalgesic animals may result in a considerable functional upregulation of $Ca_v3.2$ channels, especially at the resting membrane potential [27]. As a consequence, Ca^{2+} entry in response to each action potential and a probability of rebound discharges are increased, thus far sensitizing the NTCN neurons and contributing to the thermal hyperalgesia. In contrast, decreased functional expression of both $Ca_v3.2$ and $Ca_v3.3$ channels in the "slow" NTCN neurons of hypo- and normalgesic animals reduces neuronal excitability and Ca^{2+} entry contributing to the diminished pain sensation.

5. Conclusions

Our results demonstrate that diabetes-induced alterations in functioning of T-channels are different in the NTCN neurons expressing fast and slow T-currents and are specifically associated with different types of PDN that may underlie the variety of pain syndromes induced by type 1 diabetes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by NASU Biotechnology and Functional Genomics and Metabolomics Grants and DFFD F46.2/001 and F47/066 Grants.

References

[1] C. Gooch and D. Podwall, "The diabetic neuropathies," *Neurologist*, vol. 10, no. 6, pp. 311–322, 2004.

[2] N. A. Calcutt, J. D. Freshwater, and A. P. Mizisin, "Prevention of sensory disorders in diabetic Sprague-Dawley rats by aldose reductase inhibition or treatment with ciliary neurotrophic factor," *Diabetologia*, vol. 47, no. 4, pp. 718–724, 2004.

[3] K. K. Beiswenger, N. A. Calcutt, and A. P. Mizisin, "Dissociation of thermal hypoalgesia and epidermal denervation in streptozotocin-diabetic mice," *Neuroscience Letters*, vol. 442, no. 3, pp. 267–272, 2008.

[4] I. G. Obrosova, "Diabetic painful and insensate neuropathy: pathogenesis and potential treatments," *Neurotherapeutics*, vol. 6, no. 4, pp. 638–647, 2009.

[5] M. Dobretsov, M. M. Backonja, D. Romanovsky, and J. R. Stimers, "Animal models of diabetic neuropathic pain," *Neuromethods*, vol. 49, pp. 147–169, 2011.

[6] T.-J. Huang, N. M. Sayers, P. Fernyhough, and A. Verkhratsky, "Diabetes-induced alterations in calcium homeostasis in sensory neurones of streptozotocin-diabetic rats are restricted to lumbar ganglia and are prevented by neurotrophin-3," *Diabetologia*, vol. 45, no. 4, pp. 560–570, 2002.

[7] E. Kostyuk, N. Voitenko, I. Kruglikov et al., "Diabetes-induced changes in calcium homeostasis and the effects of calcium channel blockers in rat and mice nociceptive neurons," *Diabetologia*, vol. 44, no. 10, pp. 1302–1309, 2001.

[8] I. Kruglikov, O. Gryshchenko, L. Shutov, E. Kostyuk, P. Kostyuk, and N. Voitenko, "Diabetes-induced abnormalities in ER calcium mobilization in primary and secondary nociceptive neurons," *Pflugers Archiv European Journal of Physiology*, vol. 448, no. 4, pp. 395–401, 2004.

[9] L. Shutov, I. Kruglikov, O. Gryshchenko et al., "The effect of nimodipine on calcium homeostasis and pain sensitivity in diabetic rats," *Cellular and Molecular Neurobiology*, vol. 26, no. 7-8, pp. 1541–1557, 2006.

[10] J. N. Campbell and R. A. Meyer, "Mechanisms of neuropathic pain," *Neuron*, vol. 52, no. 1, pp. 77–92, 2006.

[11] A. Veves, M. Backonja, and R. A. Malik, "Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options," *Pain Medicine*, vol. 9, no. 6, pp. 660–674, 2008.

[12] A. I. Basbaum, D. M. Bautista, G. Scherrer, and D. Julius, "Cellular and molecular mechanisms of pain," *Cell*, vol. 139, no. 2, pp. 267–284, 2009.

[13] E. Perez-Reyes, "Molecular physiology of low-voltage-activated T-type calcium channels," *Physiological Reviews*, vol. 83, no. 1, pp. 117–161, 2003.

[14] S. Pathirathna, B. C. Brimelow, M. M. Jagodic et al., "New evidence that both T-type calcium channels and GABAA channels are responsible for the potent peripheral analgesic effects of 5α-reduced neuroactive steroids," *Pain*, vol. 114, no. 3, pp. 429–443, 2005.

[15] S. M. Todorovic, V. Jevtovic-Todorovic, A. Meyenburg et al., "Redox modulation of T-Type calcium channels in rat peripheral nociceptors," *Neuron*, vol. 31, no. 1, pp. 75–85, 2001.

[16] S. M. Todorovic, A. Meyenburg, and V. Jevtovic-Todorovic, "Mechanical and thermal antinociception in rats following systemic administration of mibefradil, a T-type calcium channel blocker," *Brain Research*, vol. 951, no. 2, pp. 336–340, 2002.

[17] E. Bourinet, A. Alloui, A. Monteil et al., "Silencing of the Cav3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception," *The EMBO Journal*, vol. 24, no. 2, pp. 315–324, 2005.

[18] S. Choi, H. S. Na, J. Kim et al., "Attenuated pain responses in mice lacking CaV3.2 T-type channels," *Genes, Brain and Behavior*, vol. 6, no. 5, pp. 425–431, 2007.

[19] S. M. Todorovic and V. Jevtovic-Todorovic, "T-type voltage-gated calcium channels as targets for the development of novel pain therapies," *British Journal of Pharmacology*, vol. 163, no. 3, pp. 484–495, 2011.

[20] R. B. Messinger, A. K. Naik, M. M. Jagodic et al., "In vivo silencing of the CaV3.2 T-type calcium channels in sensory neurons alleviates hyperalgesia in rats with streptozocin-induced diabetic neuropathy," *Pain*, vol. 145, no. 1-2, pp. 184–195, 2009.

[21] A. Dogrul, L. R. Gardell, M. H. Ossipov, F. C. Tulunay, J. Lai, and F. Porreca, "Reversal of experimental neuropathic pain by T-type calcium channel blockers," *Pain*, vol. 105, no. 1-2, pp. 159–168, 2003.

[22] M. E. Hildebrand and T. P. Snutch, "Contributions of T-type calcium channels to the pathophysiology of pain signaling," *Drug Discovery Today*, vol. 3, no. 3, pp. 335–341, 2006.

[23] T. P. Snutch and L. S. David, "T-type calcium channels: an emerging therapeutic target for the treatment of pain," *Drug Development Research*, vol. 67, no. 4, pp. 404–415, 2006.

[24] S. M. Todorovic and V. Jevtovic-Todorovic, "Regulation of T-type calcium channels in the peripheral pain pathway," *Channels*, vol. 1, no. 4, pp. 238–245, 2007.

[25] M. M. Jagodic, S. Pathirathna, M. T. Nelson et al., "Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons," *Journal of Neuroscience*, vol. 27, no. 12, pp. 3305–3316, 2007.

[26] E. M. Talley, L. L. Cribbs, J.-H. Lee, A. Daud, E. Perez-Reyes, and D. A. Bayliss, "Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels," *Journal of Neuroscience*, vol. 19, no. 6, pp. 1895–1911, 1999.

[27] E. V. Khomula, V. Y. Viatchenko-Karpinski, A. L. Borisyuk et al., "Specific functioning of Cav3.2 T-type calcium and TRPV1 channels under different types of STZ-diabetic neuropathy," *Biochimica et Biophysica Acta*, no. 5, pp. 636–649, 2013.

[28] B. Coste, M. Crest, and P. Delmas, "Pharmacological dissection and distribution of NaN/Nav1.9, T-type Ca^{2+} currents, and mechanically activated cation currents in different populations of DRG neurons," *Journal of General Physiology*, vol. 129, no. 1, pp. 57–77, 2007.

[29] C. G. Cardenas, L. P. del Mar, and R. S. Scroggs, "Variation in serotonergic inhibition of calcium channel currents in four types of rat sensory neurons differentiated by membrane properties," *Journal of Neurophysiology*, vol. 74, no. 5, pp. 1870–1879, 1995.

[30] R. S. Scroggs and A. P. Fox, "Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size," *Journal of Physiology*, vol. 445, pp. 639–658, 1992.

[31] C. L. Stucky and G. R. Lewin, "Isolectin B4-positive and -negative nociceptors are functionally distinct," *Journal of Neuroscience*, vol. 19, no. 15, pp. 6497–6505, 1999.

[32] C. J. Woolf and Q. Ma, "Nociceptors-noxious stimulus detectors," *Neuron*, vol. 55, no. 3, pp. 353–364, 2007.

[33] L. Vulchanova, T. H. Olson, L. S. Stone, M. S. Riedl, R. Elde, and C. N. Honda, "Cytotoxic targeting of isolectin IB4-binding sensory neurons," *Neuroscience*, vol. 108, no. 1, pp. 143–155, 2001.

[34] W. D. Snider and S. B. McMahon, "Tackling pain at the source: new ideas about nociceptors," *Neuron*, vol. 20, no. 4, pp. 629–632, 1998.

[35] C.-L. Chen, D. C. Broom, Y. Liu et al., "Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain," *Neuron*, vol. 49, no. 3, pp. 365–377, 2006.

[36] K. Hargreaves, R. Dubner, F. Brown, C. Flores, and J. Joris, "A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia," *Pain*, vol. 32, no. 1, pp. 77–88, 1988.

[37] M. B. Gerke and M. B. Plenderleith, "Binding sites for the plant lectin Bandeiraea simplicifolia I-isolectin B4 are expressed by nociceptive primary sensory neurones," *Brain Research*, vol. 911, no. 1, pp. 101–104, 2001.

[38] J.-H. Lee, J. C. Gomora, L. L. Cribbs, and E. Perez-Reyes, "Nickel block of three cloned T-type calcium channels: low concentrations selectively block α1H," *Biophysical Journal*, vol. 77, no. 6, pp. 3034–3042, 1999.

[39] A. Tscherter, F. David, T. Ivanova et al., "Minimal alterations in T-type calcium channel gating markedly modify physiological firing dynamics," *Journal of Physiology*, vol. 589, no. 7, pp. 1707–1724, 2011.

[40] J. Yue, L. Liu, Z. Liu, B. Shu, and Y. Zhang, "Upregulation of T-type Ca^{2+} channels in primary sensory neurons in spinal nerve injury," *Spine*, vol. 38, no. 6, pp. 463–470, 2013.

[41] K. E. Rose, N. Lunardi, A. Boscolo et al., "Immunohistological demonstration of CaV3.2 T-type voltage-gated calcium channel expression in soma of dorsal root ganglion neurons and peripheral axons of rat and mouse," *Neuroscience*, vol. 250, pp. 263–274, 2013.

[42] X. Zhong, J. R. Liu, J. W. Kyle, D. A. Hanck, and W. S. Agnew, "A profile of alternative RNA splicing and transcript variation of CACNA1H, a human T-channel gene candidate for idiopathic generalized epilepsies," *Human Molecular Genetics*, vol. 15, no. 9, pp. 1497–1512, 2006.

[43] K. E. Hall, A. A. F. Anders, and J. W. Wiley, "Voltage-dependent calcium currents are enhanced in dorsal root ganglion neurones from the Bio Bred/Worchester diabetic rat," *Journal of Physiology*, vol. 486, no. 2, pp. 313–322, 1995.

[44] K. E. Hall, J. Liu, A. A. F. Sima, and J. W. Wiley, "Impaired inhibitory G-protein function contributes to increased calcium currents in rats with diabetic neuropathy," *Journal of Neurophysiology*, vol. 86, no. 2, pp. 760–770, 2001.

[45] J. R. Latham, S. Pathirathna, M. M. Jagodic et al., "Selective T-type calcium channel blockade alleviates hyperalgesia in ob/ob mice," *Diabetes*, vol. 58, no. 11, pp. 2656–2665, 2009.

[46] W. Choe, R. B. Messinger, E. Leach et al., "TTA-P2 is a potent and selective blocker of T-type calcium channels in rat sensory neurons and a novel antinociceptive agent," *Molecular Pharmacology*, vol. 80, no. 5, pp. 900–910, 2011.

[47] M. M. Jagodic, S. Pathirathna, P. M. Joksovic et al., "Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve," *Journal of Neurophysiology*, vol. 99, no. 6, pp. 3151–3156, 2008.

[48] L. A. Swayne and E. Bourinet, "Voltage-gated calcium channels in chronic pain: emerging role of alternative splicing," *Pflugers Archiv European Journal of Physiology*, vol. 456, no. 3, pp. 459–466, 2008.

[49] M. T. Nelson, P. M. Joksovic, E. Perez-Reyes, and S. M. Todorovic, "The endogenous redox agent L-cysteine induces T-type Ca^{2+} channel-dependent sensitization of a novel subpopulation of rat peripheral nociceptors," *Journal of Neuroscience*, vol. 25, no. 38, pp. 8766–8775, 2005.

[50] A. S. Kozlov, F. McKenna, J.-H. Lee et al., "Distinct kinetics of cloned T-type Ca^{2+} channels lead to differential Ca^{2+} entry and frequency-dependence during mock action potentials," *European Journal of Neuroscience*, vol. 11, no. 12, pp. 4149–4158, 1999.

[51] R. C. H. Warre, N. C. L. McNaughton, and A. D. Randall, "Differential discrimination of fast and slow synaptic waveforms by two low-voltage-activated calcium channels," *Neuroscience*, vol. 110, no. 2, pp. 375–388, 2002.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2014, Article ID 628531, 11 pages
http://dx.doi.org/10.1155/2014/628531

Research Article

Dual Effect of Exogenous Nitric Oxide on Neuronal Excitability in Rat Substantia Gelatinosa Neurons

A-Reum Park,[1] Hae In Lee,[1] Dejidnorov Semjid,[1] Do Kyung Kim,[2] and Sang Woo Chun[1]

[1] *Department of Oral Physiology, College of Dentistry, Institute of Wonkwang Biomaterial and Implant, Wonkwang University, 344-2 Shinyong Dong, Iksan 570-749, Republic of Korea*
[2] *Oral Biology Research Institute, Chosun University School of Dentistry, Gwangju 501-759, Republic of Korea*

Correspondence should be addressed to Sang Woo Chun; physio1@wku.ac.kr

Received 19 September 2013; Revised 26 November 2013; Accepted 27 November 2013; Published 8 January 2014

Academic Editor: Dong-ho Youn

Nitric oxide (NO) is an important signaling molecule involved in nociceptive transmission. It can induce analgesic and hyperalgesic effects in the central nervous system. In this study, patch-clamp recording was used to investigate the effect of NO on neuronal excitability in substantia gelatinosa (SG) neurons of the spinal cord. Different concentrations of sodium nitroprusside (SNP; NO donor) induced a dual effect on the excitability of neuronal membrane: 1 mM of SNP evoked membrane hyperpolarization and an outward current, whereas 10 μM induced depolarization of the membrane and an inward current. These effects were prevented by hemoglobin and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt (c-PTIO) (NO scavengers), phenyl *N*-tert-butylnitrone (PBN; nonspecific reactive oxygen species scavenger), and through inhibition of soluble guanylyl cyclase (sGC). Pretreatment with n-ethylmaleimide (NEM; thiol-alkylating agent) also decreased effects of both 1 mM and 10 μM SNP, suggesting that these responses were mediated by direct S-nitrosylation. Charybdotoxin (CTX) and tetraethylammonium (TEA) (large-conductance Ca^{2+}-activated K^+ channel blockers) and glybenclamide (ATP-sensitive K^+ channel blocker) decreased SNP-induced hyperpolarization. La^{3+} (nonspecific cation channel blocker), but not Cs^+ (hyperpolarization-activated K^+ channel blocker), blocked SNP-induced membrane depolarization. In conclusion, NO dually affects neuronal excitability in a concentration-dependent manner via modification of various K^+ channels.

1. Introduction

Nitric oxide (NO) is a pivotal signaling molecule involved in many diverse developmental and physiological processes in the mammalian nervous system [1–3]. NO is biosynthesized from L-arginine by specific neuronal and non-neuronal forms of NO synthase [4, 5]. NO donors as well as endogenously produced NO play a role in many physiological processes, including smooth muscle relaxation, cellular proliferation, apoptosis, neurotransmitter release, and cell differentiation [6]. NO-induced effects are commonly mediated through the following processes: increased cGMP production upon activation of NO-sensitive soluble guanylyl cyclase (sGC), S-nitrosylation, tyrosine nitration, and NO interaction with superoxide ($O_2^{\bullet-}$) to form peroxynitrite ($ONOO^-$) [1, 7, 8].

Oxidative stress due to reactive oxygen species (ROS) such as $O_2^{\bullet-}$, hydrogen peroxide (H_2O_2), NO, and $ONOO^-$ interferes with normal cell function and can cause cell damage. Moreover, ROS is associated with chronic pain, particularly neuropathic and inflammatory pain [9, 10]. NO has a dual role in the regulation of pain processes; it can mediate a nociceptive or induce an antinociceptive effect. Some studies suggest that spinal NO is involved in the potentiation of nociception. For example, it has been demonstrated that nerve injury- or tissue inflammation-induced mechanical hypersensitivity is reduced in nNOS knockout mice and by intrathecal administration of nNOS inhibitors [11–13]. Furthermore, NO, produced in the NOS-containing spinal cord neurons, plays a pivotal role in chronic pain [14, 15].

In contrast, other studies have shown that administration of NO donors can induce antinociceptive effects. For

example, L-arginine and 3-morpholinosydnonimine (SIN-1; NO donor), administered intracerebroventricularly to mice, cause antinociception [16]. Intraplantar injection of sodium nitroprusside (SNP), a substance which nonenzymatically releases NO, also causes antinociception in rats [17].

The substantia gelatinosa (SG) of the dorsal horn is the first site of synaptic transmission in the nociceptive pathway, and it is an area vital for the integration and modulation of the peripheral nociceptive input. Understanding neuronal excitability in this area is fundamental to enhance our knowledge on nociceptive neurotransmission. However, despite many reports on the importance of NO in nociceptive processing in the spinal cord, the effect of NO on the excitability of spinal cord dorsal horn neurons remains unclear. In this study, the effect of different concentrations of NO on the membrane potential of SG neurons was investigated using patch-clamp recordings from transverse slices of the spinal cord.

2. Materials and Methods

2.1. Spinal Cord Slice Preparation. Sprague-Dawley rats (14–18 days old) were first anesthetized with ether. The procedures were approved by the University of Wonkwang Committee on Ethics in the Care and Use of Laboratory Animals (WKU09-076). Lumbosacral laminectomy was performed following intraperitoneal administration of 25% urethane. The spinal cord at spinal level L1-S3 was removed and placed in a preoxygenated solution at 1-2°C. Transverse spinal slices, 350 μm thick, were prepared using a vibroslicer (752M, Campden Instruments, Loughborough, UK) and incubated at 32°C for a recovery period of at least 1 h. Afterwards, slices were transferred to a recording chamber mounted on a upright microscope.

2.2. Solution and Drugs. The dissecting solution for the spinal cord slice preparation was composed of (in mM) 252 Sucrose, 2.5 KCl, 0.1 $CaCl_2$, 2 $MgCl_2$, 10 Glucose, 26 $NaHCO_3$, and 1.25 NaH_2PO_4. The extracellular fluid used for the patch-clamp recording contained (in mM) 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose. It was continually aerated with 95% O_2/5% CO_2, which kept the pH at approximately 7.4. The pipette (internal) solution contained (in mM) 150 K-Glu, 10 Hepes, 5 KCl, 0.1 EGTA, 2 Mg-ATP, and 0.3 Na GTP. The pH was adjusted to 7.3 by KOH. 1H-[1,2,4]oxadiazole[4,3-α]quinoxaline-1-one (ODQ) and glibenclamide were dissolved in DMSO to prepare a stock solution. SNP, hemoglobin (Hb), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt (c-PTIO), phenyl *N*-tert-butylnitrone (PBN), ODQ, lanthanum chloride, cesium chloride, charybdotoxin (CTX), tetraethylammonium (TEA), glibenclamide, apamin, and n-ethylmaleimide (NEM) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Patch-Clamp Recording. Microelectrodes were prepared from capillary glass tubes (TW150-3, WPI, USA) using a microelectrode pipette puller (PP830, Narishige, Japan). Patch pipettes, filled with the pipette solutions, were used at a resistance ranging from 6 to 8 MΩ. The substantia gelatinosa of the spinal cord was viewed with an upright microscope (BX50WI, Olympus, Japan). Membrane potential and current were recorded using an Axopatch 200B (Axon Instruments, USA) amplifier that was connected to a computer using an A/D converter (Digidata 1322A, Axon Instruments, USA). Membrane potential recording and data analyses were performed using pClamp software (Version 9.0, Axon Instruments, USA). Generated currents were filtered with a low-pass 8-pole Bessel filter at 2 kHz. All experiments were performed at room temperature (22 ± 1°C).

2.4. Fluorescence Imaging. For detection of nitric oxide, spinal cord slices were incubated with 10 μM of 4-amino-5-methylamino-2,7-difluorofluorescein diacetate (DAF-FM DA) for 30 minutes at 32°C. The slices were examined on an inverted fluorescence microscope (LSM 510, Carl Zeiss, Germany). Excitation wavelength was 488 nm, and emission was measured at 515 to 565 nm. A time series was used to record images every 30 s.

2.5. Data Analysis. Differences in drug effects were analyzed using independent t-test and were considered significant when $P < 0.05$. Data are expressed as mean ± SEM.

3. Results

3.1. Effects of SNP (1 mM and 10 μM) on the Membrane Excitability in Substantia Gelatinosa Neurons of the Spinal Cord. During current-clamp recording, a high concentration of SNP (1 mM) induced membrane hyperpolarization (−7.5 ± 1.0 mV, $n = 62$), whereas a low concentration (10 μM) induced membrane depolarization (4.4 ± 0.7 mV, $n = 32$) (Figures 1(a) and 1(c)). When voltage clamp recording was performed at a holding potential of −60 mV, SNP (1 mM) induced an outward current (5.7 ± 0.6 pA, $n = 50$), whereas SNP (10 μM) induced an inward current (−4.8 ± 1.1 pA, $n = 14$) (Figures 1(b) and 1(d)). This suggests that SNP can elicit dual effects on the membrane excitability of SG neurons in a concentration-dependent manner.

3.2. Effects of NO Scavengers on SNP-Induced Membrane Potential Changes. We next investigated the effects of NO scavengers to determine whether the SNP-induced changes in membrane potential were due to the release of NO from the donor. SNP (1 mM)-induced hyperpolarization is significantly reduced in the presence of the NO scavengers, Hb (50 μM) (−4.5 ± 0.9 mV, $n = 8$, $P < 0.05$) and c-PTIO (200 μM) (−3.7 ± 0.4 mV, $n = 8$, $P < 0.01$) (Figures 2(a), 2(c), and 2(g)). Furthermore, pretreatment with Hb (0.6 ± 0.6 mV, $n = 6$, $P < 0.001$) and c-PTIO (1.4 ± 0.5 mV, $n = 5$, $P < 0.01$) significantly inhibited SNP (10 μM)-mediated depolarization (Figures 2(b), 2(d), and 2(g)). Pretreatment with PBN, the nonspecific ROS scavenger, significantly reduced SNP-induced hyperpolarization (−2.2 ± 1.6 mV, $n = 5$, $P < 0.05$) (Figures 2(e) and 2(g)) as well as SNP-induced depolarization (1.4 ± 0.4 mV, $n = 5$, $P < 0.001$) (Figures 2(f) and 2(g)). These

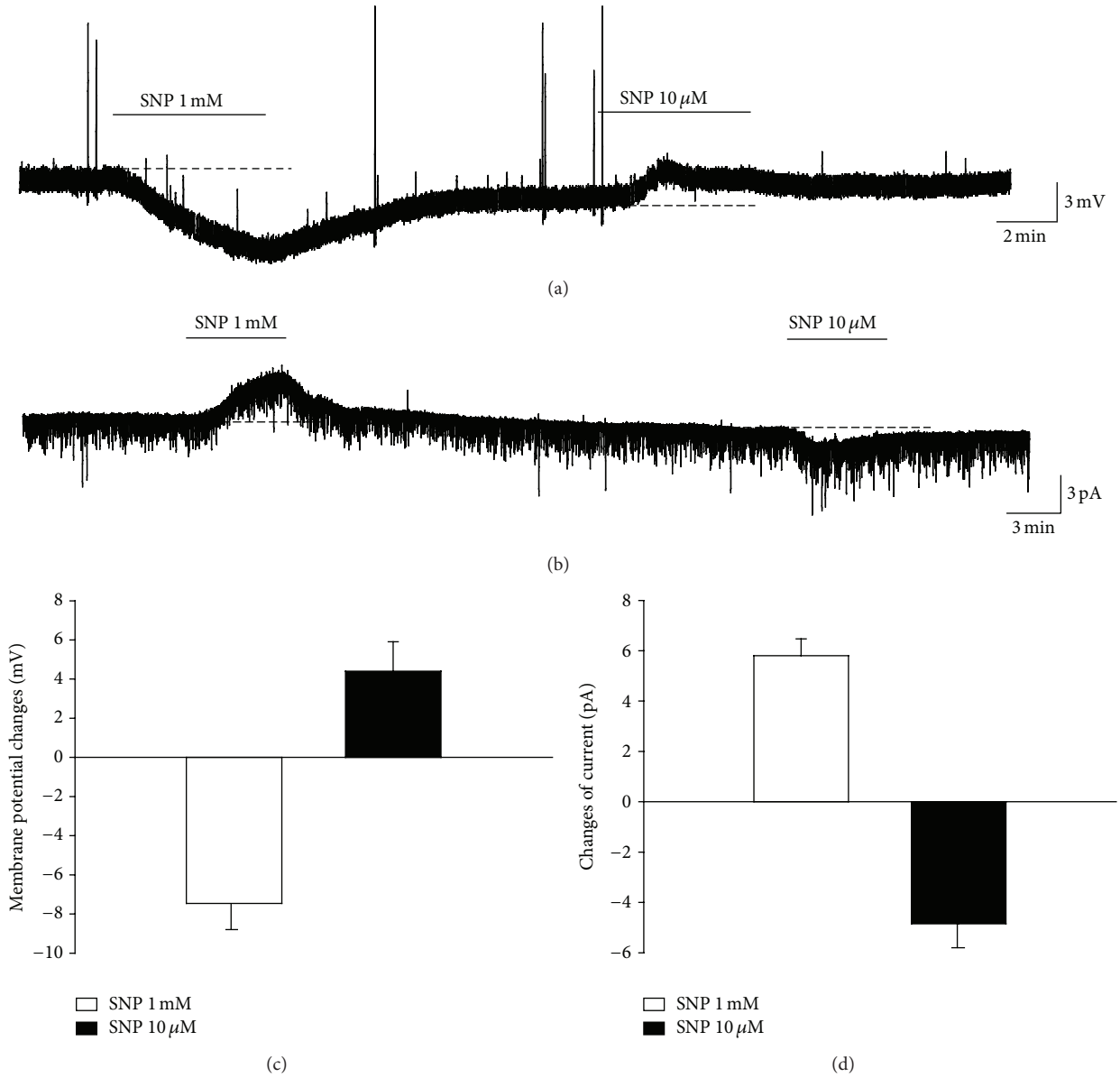


Figure 1: Effect of 1 mM and 10 μM SNP on the membrane excitability in substantia gelatinosa (SG) neurons of the spinal cord. (a) Current-clamp recording of membrane potentials of SG neurons showing the dual effect of SNP. SNP (1 mM) induced membrane hyperpolarization, whereas SNP (10 μM) elicited membrane depolarization. (b) Representative current traces of SG neurons recorded at a holding potential of −60 mV. SNP (1 mM) induced an outward current, whereas SNP (10 μM) induced an inward current. (c) Bar graphs show the membrane potential changes evoked by different concentrations of SNP. (d) Bar graphs show the amplitude of current changes induced by different concentrations of SNP. Mean ± SEM.

results suggest that NO is released by SNP, which in turn induces the changes in membrane excitability of SG neurons.

3.3. Fluorescence Response of NO in DAF-FM DA-Loaded SG Neurons. The effect of SNP on NO production was determined using the cell-permeable fluorescent probe, DAF-FM DA. SNP is a donor of NO; thus, it can release NO, which then reacts with DAF-FM to produce fluorescence. Figure 3 shows changes in intracellular fluorescence intensity over a time series of images taken every 30 s. Intracellular NO production was induced during SNP perfusion for 5 min. Increased NO production (128.0 ± 6.1%, $n = 12$) was inhibited by the NO scavenger, Hb (50 μM) (92.0 ± 0.5%, $n = 5$, $P < 0.05$) (Figures 3(a), 3(b), and 3(d)), and the ROS scavenger, PBN (2 mM) (95.1 ± 2.1%, $n = 7$, $P < 0.05$) (Figures 3(a), 3(c), and 3(e)).

3.4. Involvement of Soluble Guanylyl Cyclase in the SNP-Induced Response. NO has been shown to activate sGC, leading to an increase in cGMP levels. Thus, to determine whether the effect of SNP was mediated by the activation of sGC, ODQ (40 μM), a selective sGC inhibitor, was used

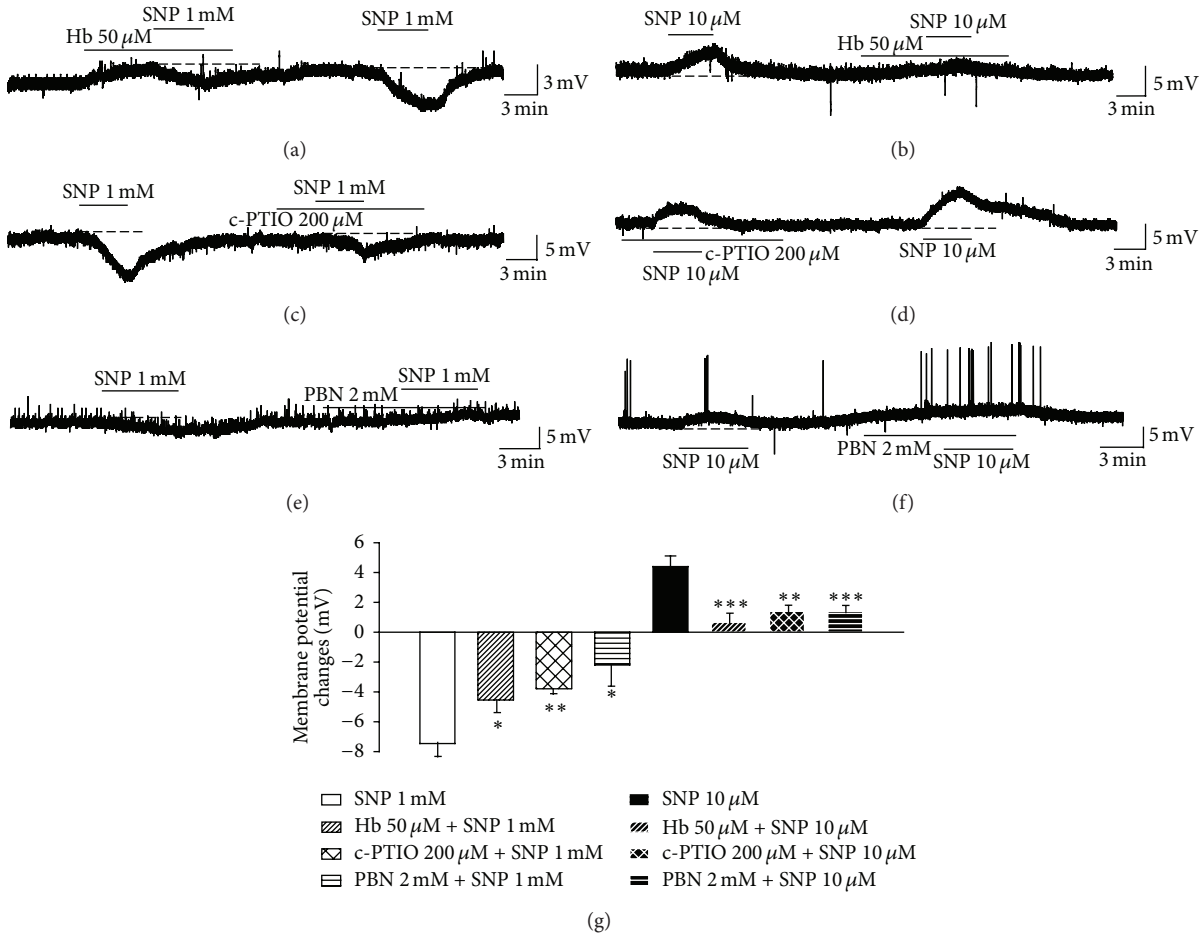


Figure 2: Effect of the NO scavengers on SNP-induced membrane potential changes. SNP (1 mM)-induced hyperpolarization was decreased by pretreatment with Hb (a) and c-PTIO (c). Hb (b) and c-PTIO (d) decreased SNP (10 μM)-induced depolarization. (e) Pretreatment with PBN inhibited SNP (1 mM)-induced hyperpolarization. (f) PBN reduced SNP (10 μM)-induced depolarization. (g) Summary of data obtained under the control condition of SNP-induced responses and pretreatment with Hb, c-PTIO, and PBN. *Values are significantly different from the control (SNP), based on independent t-test analysis ($P < 0.05$), $^{**}P < 0.01$, $^{***}P < 0.001$. Mean ± SEM.

in the presence of both concentrations of SNP (1 mM and 10 μM). Pretreatment with ODQ inhibited SNP (1 mM)-induced membrane hyperpolarization (−2.5 ± 0.9 mV, $n = 6$, $P < 0.01$) (Figures 4(a) and 4(c)) as well as SNP (10 μM)-induced depolarization (1.5 ± 0.4 mV, $n = 6$, $P < 0.001$) (Figures 4(b) and 4(d)). These results suggest that the SNP-activated signaling pathway is dependent upon sGC.

3.5. Effect of a Thiol-Modifying Agent on the SNP-Induced Responses. A known alternative pathway for the biological effects of NO is the direct S-nitrosylation of critical cysteine thiol group(s) of target proteins [18]. To determine whether the SNP-evoked responses involved the direct modulation of membrane proteins by NO, we examined the effect of NEM, which blocks sulfhydryl groups, on SG neurons. Membrane hyperpolarization induced by SNP (1 mM) was significantly decreased by pretreatment with NEM (−4.4 ± 0.8 mV, $n = 5$, $P < 0.05$) (Figures 5(a) and 5(c)). Depolarization by SNP (10 μM) was also significantly inhibited by the presence of NEM (−0.1 ± 1.4 mV, $n = 7$, $P < 0.05$) (Figures 5(b) and 5(d)). Similar results were observed for voltage clamp recordings. An inward current induced by SNP (10 μM) and an outward current induced by SNP (1 mM) were inhibited by pretreatment with NEM (data not shown). These results indicate that SNP-induced responses are mediated via direct S-nitrosylation of channel protein.

3.6. Involvement of Various K^+ Channels on SNP-Induced Membrane Hyperpolarization. Different mechanisms of NO-dependent effects have been reported in the literature, including the direct activation of K^+ channels [1, 3, 19]. Therefore, we next determined the ion channels involved in the SNP-induced hyperpolarization. Significant inhibition of hyperpolarization was observed in the presence of CTX (−4.5 ± 0.7 mV, $n = 6$, $P < 0.05$) (Figures 6(a) and 6(e)) and TEA, large-conductance Ca^{2+}-activated K^+ (BK) channel blockers (−3.3 ± 0.7 mV, $n = 10$, $P < 0.01$) (Figures 6(b) and 6(e)). However, it was not significantly inhibited in the presence of apamin, small-conductance Ca^{2+}-activated K^+ (SK) channel blocker (−5.8 ± 0.7 mV, $n = 6$) (Figures 6(c) and 6(e)). In

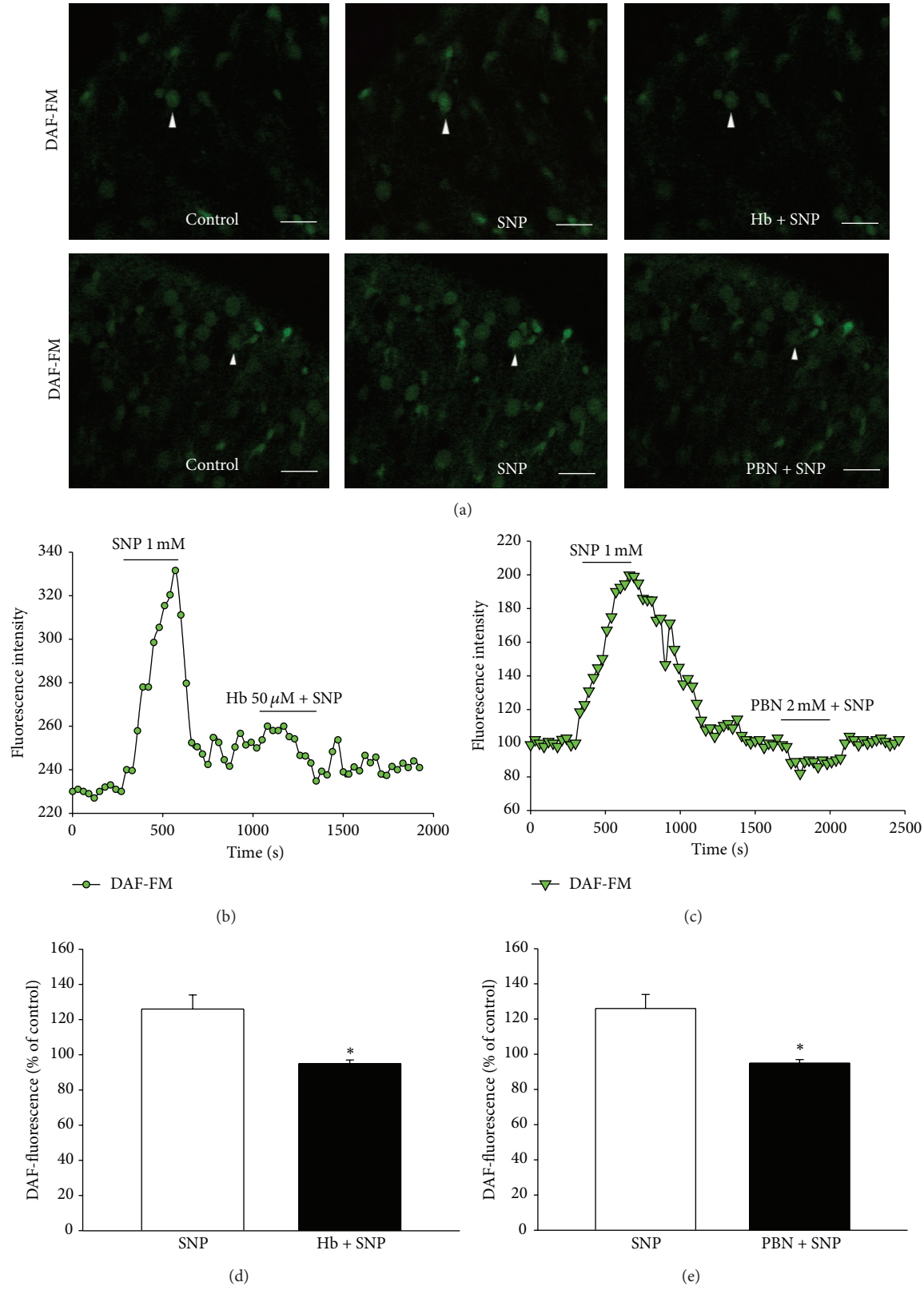


Figure 3: Fluorescence response of NO in DAF-FM DA-loaded spinal cord slices. (a) After addition of SNP (1 mM), fluorescence intensity increased. Hemoglobin (50 μM) (upper) and PBN (2 mM) (lower) prevented the NO-induced fluorescence increase (scale bars: 50 μm). ((b), (c)) Obtained images during the time series were shown for changes in fluorescence intensity within the regions of interest (ROI) (arrows indicate ROI). ((d), (e)) The results were quantitatively analyzed as percent units of DAF fluorescence of the control. *Values are significantly different from the control (SNP), based on independent t-test analysis ($P < 0.05$). Mean ± SEM.

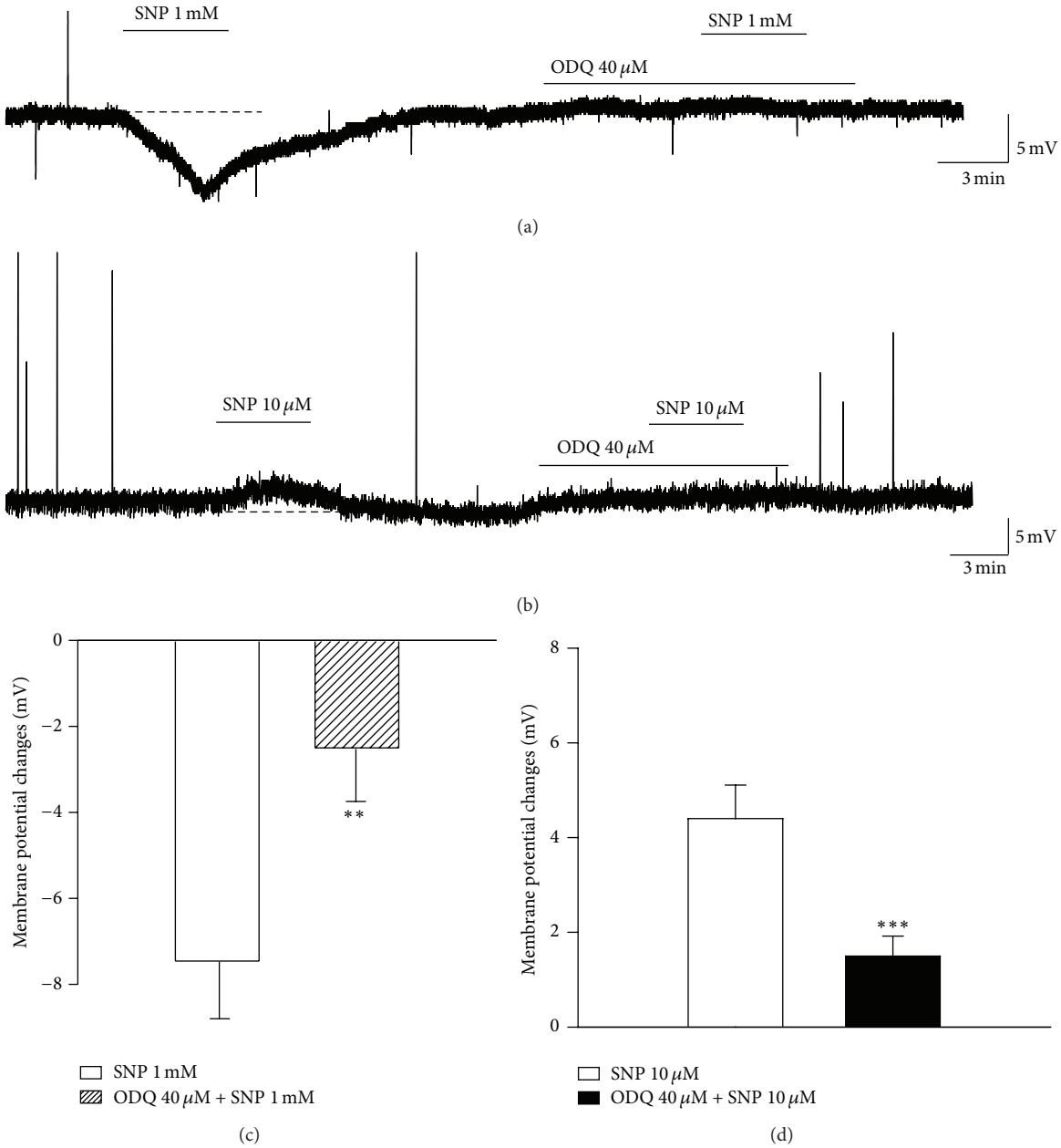


Figure 4: Soluble guanylyl cyclase is involved in the SNP-induced responses. (a) SNP (1 mM)-induced membrane hyperpolarization in SG neurons was blocked by ODQ (40 μM). (b) Membrane depolarization by SNP (10 μM) was inhibited by pretreated with ODQ. (c) Summary data obtained under the control condition of 1 mM SNP-induced hyperpolarization and pretreatment with ODQ. (d) Summary data obtained under the control condition of 10 μM SNP-induced depolarization and pretreatment with ODQ. **Values are significantly different from the control (SNP), based on independent t-test analysis ($P < 0.01$), $^{***}P < 0.001$. Mean ± SEM.

addition, membrane hyperpolarization was also significantly inhibited by application of glibenclamide, an ATP-sensitive K^+ (K_{ATP}) channel blocker (−3.3 ± 0.5 mV, $n = 6$, $P < 0.001$) (Figures 6(d) and 6(e)). These observations suggest that NO generated its effect through the activation of various K^+ channels.

3.7. Involvement of a Nonspecific Cation Channel in Membrane Depolarization Induced by SNP. Recently, it was reported that SNP depolarizes the membrane potential of SG neurons and that this effect is inhibited by the presence of 1 mM Cs^+ [20]. Based on this report, we tested whether SNP-induced depolarization was caused by the activation of hyperpolarization-activated K^+ channel. Depolarization induced by a low concentration of SNP (10 μM) was not inhibited by the presence of 1 mM Cs^+ (4.5 ± 0.7 mV, $n = 7$) (Figures 7(a) and 7(c)). However, it was significantly blocked by the presence of a nonspecific cation channel blocker, lanthanum (1.4 ± 0.5 mV, $n = 5$, $P < 0.01$) (Figures 7(b)

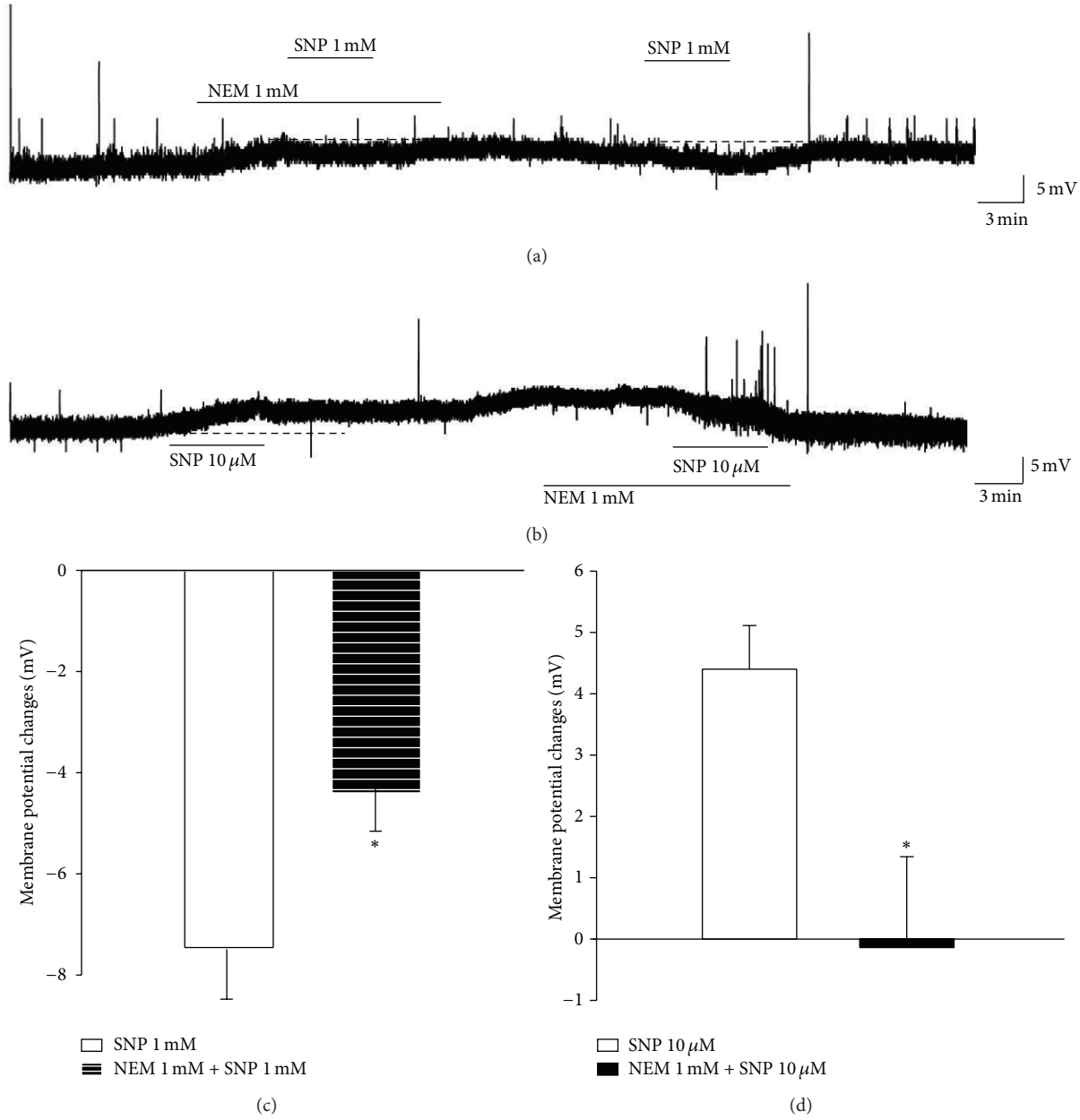


Figure 5: Effect of NEM, a thiol-modifying agent, on SNP-induced membrane potential changes. (a) Membrane hyperpolarization, induced by SNP (1 mM), was decreased by the presence of NEM. (b) SNP (10 μM)-induced depolarization was decreased by the application of NEM. (c) Summary of data obtained under the control condition of SNP-induced hyperpolarization and pretreatment with NEM. (d) Summary of data obtained under the control condition of SNP-induced depolarization and pretreatment with NEM. *Values are significantly different from the control (SNP), based on independent t-test analysis ($P < 0.05$). Mean ± SEM.

and 7(c)). These results suggest that depolarization induced by a low concentration of SNP (10 μM) did not involve hyperpolarization-activated K^+ channels but instead involved activation of a nonspecific cation channel.

4. Discussion

NO donors as well as endogenously produced NO exert various physiological effects, including smooth muscle relaxation, apoptosis, neurotransmitter release, and neurotoxicity [6]. NO is produced in the spinal dorsal horn neurons in response to extensive nociceptive input thereby contributing to central sensitization and persistent pain [21, 22].

Recently, a dual effect of NO on pain transmission was reported. Kawabata et al. [23] observed that NO induces a nociceptive or antinociceptive effect in a dose-dependent manner in mice. These authors demonstrated that injection of a low dose of L-arginine enhanced the nociceptive response, whereas administration of a high dose suppressed the nociceptive effect. In contrast, Li and Qi

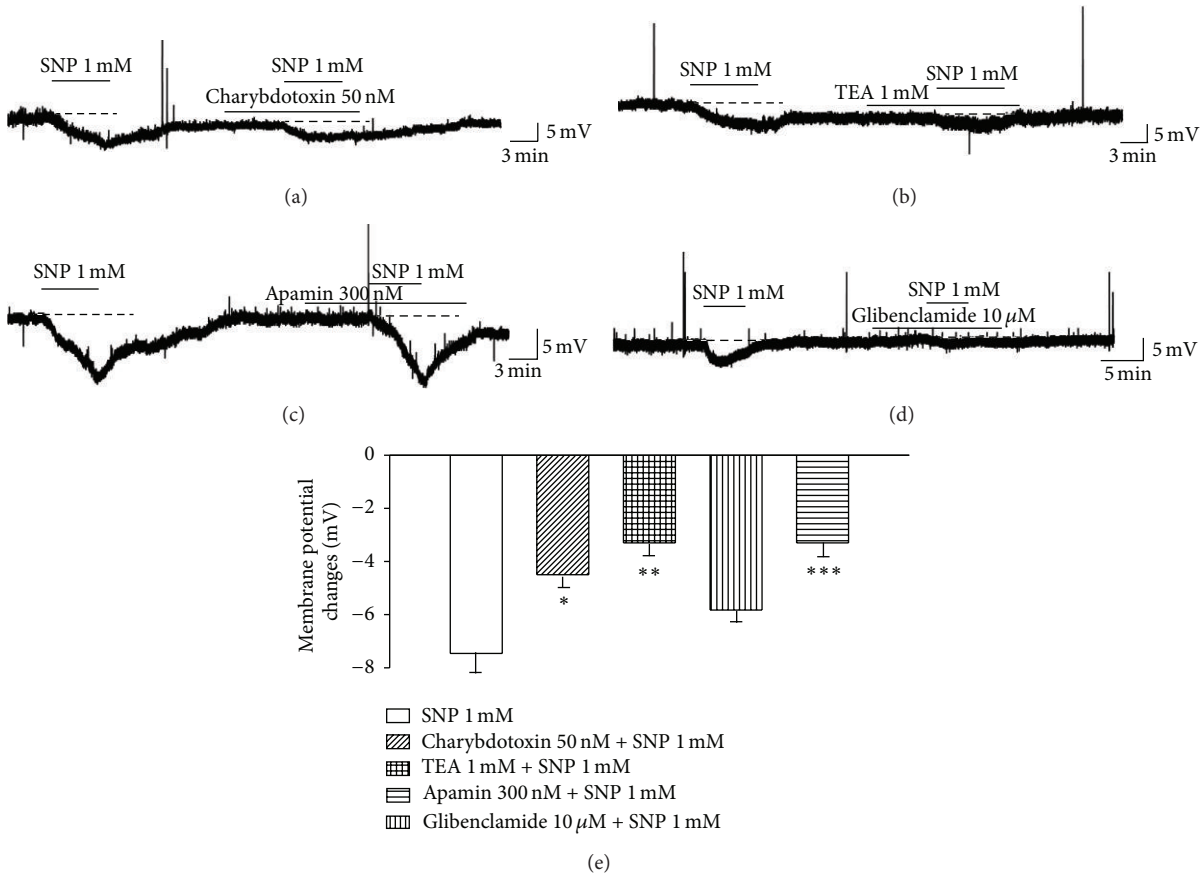


Figure 6: Involvement of various K^+ channels in SNP-induced membrane hyperpolarization. ((a), (b)) Changes in membrane potential evoked by SNP (1 mM) were significantly inhibited by CTX and TEA, BK channel blockers. (c) Membrane hyperpolarization was not significantly inhibited by the presence of apamin, a SK channel blocker. (d) Membrane hyperpolarization was inhibited by application of glibenclamide, a K_{ATP} channel blocker. (e) Bar graphs show the membrane potential changes elicited by application of various K^+ channel blockers. *Values are significantly different from the control (SNP), based on independent t-test analysis ($P < 0.05$), $^{**}P < 0.01$, $^{***}P < 0.001$. Mean ± SEM.

[24] demonstrated that intrathecal administration of low doses of L-arginine inhibited the nociceptive responses evoked by the intraplantar injection of formalin in rats, whereas high doses of the NO precursor increased this response. Furthermore, using a model of neuropathic pain in rats, Sousa and Prado [25] showed that intrathecal administration of 3-morpholinosydnonimine (SIN-1), a NO donor, produces a dual dose-dependent effect. These authors reported that low intrathecal doses of SIN-1 reduced the mechanical allodynia evoked by sciatic nerve ligation, whereas higher doses enhanced the allodynia or had no effect.

Pehl and Schmid [26] investigated the effects of different NO donors on spontaneously active neurons in the rat spinal cord using extracellular recording. They reported that NO causes direct excitation or inhibition of the electrical activity of spinal neurons. Discrepancies might be because of the differences regarding the doses of NO donors, the model used for pain evaluation, and experimental animal used in the studies [3]. Results, similar to those mentioned above, were also demonstrated in the present study, whereby application of different SNP concentrations produced a dual effect on the membrane potential of the SG neurons (Figure 1).

ROS such as $O_2^{\bullet-}$, H_2O_2, NO, and $ONOO^-$ are closely related to central sensitization [9, 10]. This study explored whether ROS are involved in the SNP-induced changes in neuronal excitability of SG neurons, produced by each concentration of SNP (1 mM or 10 μM), by applying a strong ROS scavenger, PBN. Application of PBN significantly blocked the response evoked by both concentrations of SNP (Figure 2). It seems possible that NO can react with endogenously generated $O_2^{\bullet-}$ to produce highly toxic $ONOO^-$. $ONOO^-$ has been proposed as a converged downstream molecule of $O_2^{\bullet-}$ and NO in persistent pain conditions [12]. In this study, we did not use an $ONOO^-$ decomposition catalyst to verify whether $ONOO^-$ influences SNP-induced responses. Therefore, we cannot exclude the possibility that $ONOO^-$ can modulate the excitability of SG neurons. However, Kim et al. [22] demonstrated that NO and $O_2^{\bullet-}$ operate independently, while both are contributing to the same persistent pain.

Several fluorescent probes have been designed to measure NO in biological samples [27]. The most widely used and best

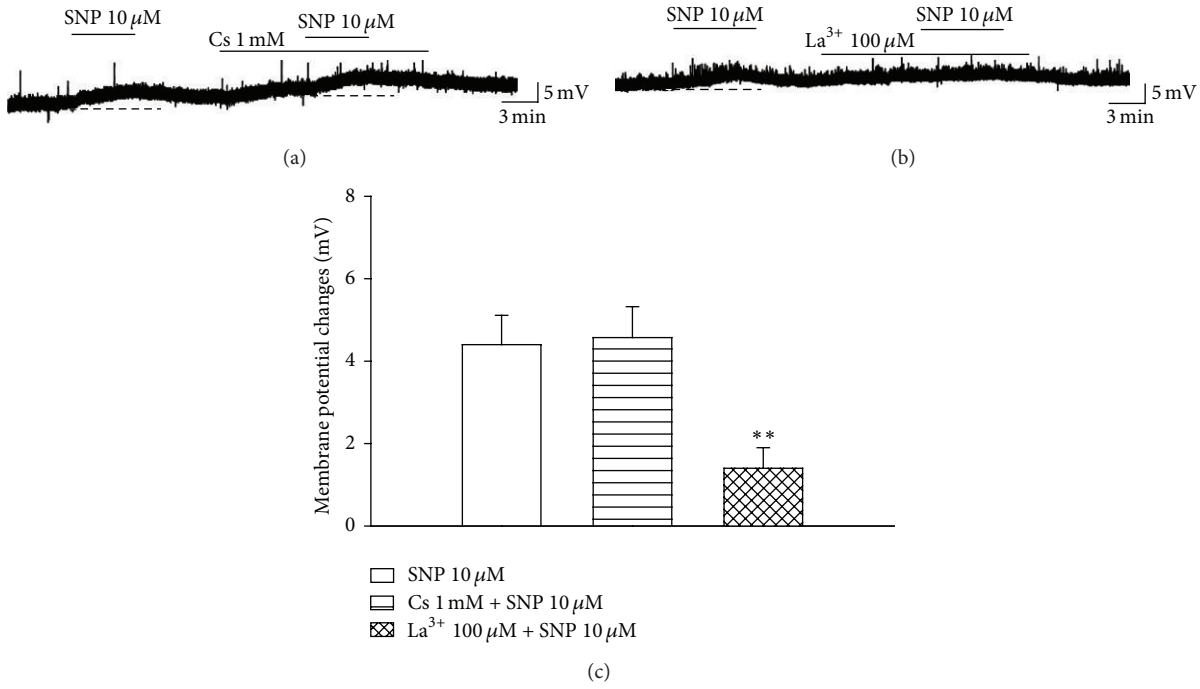


Figure 7: Involvement of a nonspecific cation channel in the membrane depolarization induced by SNP. (a) Membrane depolarization evoked by SNP (10 μM) was not inhibited by the presence of 1 mM Cs^+. (b) Depolarization evoked by SNP was significantly inhibited by La^{3+}, a nonspecific cation channel blocker. (c) Bar graphs show the membrane potential changes induced by pretreatment with Cs^+ and La^{3+}. **Values are significantly different from the control (SNP), based on independent t-test analysis ($P < 0.01$). Mean ± SEM.

characterized probes are 4,5-diaminofluorescein (DAF-2) and 4-amino-5-methylamino-2,7-difluorofluorescein (DAF-FM), both of which react with NO to form green fluorescent triazole products [28]. In this study, we confirmed the presence of SNP-induced intracellular NO production using DAF-FM. As shown in Figure 3, NO production was increased by addition of SNP in the spinal cord slices. Similar to our finding, it was previously reported that the fluorescence of DAF-FM increases in a dose- and time-dependent manner upon incubation with SNP [29]. The SNP-induced fluorescence increase observed in this study was reduced by Hb, a NO scavenger (Figures 3(a), 3(b), and 3(d)). The scavenging effect of Hb on NO has been demonstrated in several experiments [30, 31]. Moreover, we successfully used PBN, a ROS scavenger, to inhibit NO activity. Similar to a previous study [22], this result demonstrates that NO-induced fluorescence is prevented by PBN.

NO activates guanylate cyclase, which is responsible for an increase in intracellular levels of cGMP. Sousa and Prado [25] demonstrated that pretreatment with ODQ, a selective sGC inhibitor, practically abolishes the antinociceptive and pronociceptive effect mediated by an intrathecally applied NO donor. On the basis of their findings, in this study, ODQ was applied to each concentration of SNP to investigate the involvement of the NO-cGMP signaling pathway. Similar to previous reports, application of ODQ significantly blocked the response evoked by both concentrations of SNP (Figure 4). These findings demonstrate that SNP mediates its effect through a NO/sGC/cGMP pathway.

Besides activating the indirect cGMP-signaling pathway, NO can also directly modify channel proteins by S-nitrosylation [1, 18]. S-Nitrosylation is emerging as an important form of posttranslational modification of ion channels. It provides a route by which NO can regulate electrical activity without stimulating production of cGMP. Kawano et al. [18] reported that nitric oxide activates K_{ATP} channels in mammalian sensory neurons by direct S-nitrosylation. They showed that inhibition of sGC and PKG failed to block this activation by NO. In addition, they reported that NO activation of K_{ATP} currents is inhibited by thiol-alkylating agents, which demonstrates that S-nitrosylation is needed for NO action. In the present study, to determine whether SNP can directly modulate SG neurons through S-nitrosylation, NEM was applied as an S-nitrosylation blocker (Figure 5). The responses induced by both concentrations of SNP were significantly inhibited by NEM. These findings suggest that SNP mediates its effects via direct S-nitrosylation of membrane proteins in SG neurons.

K^+ channel activation may be elicited by both NO and/or NO redox forms. Both PKG and S-nitrosylation enhance the activity of BK channels. In addition, cGMP modulates the activity of a delayed rectifier K^+ channel and K_{ATP} channels through activation of PKG [1, 32–34]. To test whether NO activates K^+ channels to induce changes in membrane potential, various K^+ channel blockers were applied. Hyperpolarization evoked by SNP (1 mM) was significantly inhibited by pretreatment with CTX and TEA, BK channel blockers, and glibenclamide, a specific K_{ATP} channel blocker, but was

not altered by pretreatment with apamin, a SK channel blocker (Figure 6). These findings indicate that NO-induced membrane hyperpolarization involves the activation of both BK, and K_{ATP} channel.

Kim et al. [20] observed that application of SNP (500 μM) induced membrane depolarization in SG neurons and reported that this effect was elicited by a hyperpolarization-activated inward current. On the basis of this finding, we tested whether SNP (10 μM)-induced depolarization was caused by the activation of a hyperpolarization-activated K^+ channel by pretreating SG neurons with Cs^+. However, membrane depolarization evoked by a low concentration of SNP was not affected by pretreatment with Cs^+. Similar to our study, Sun et al. [35] demonstrated that peripheral ZD7288, a hyperpolarization-activated K^+ channel blocker, blocked neuropathic pain while intrathecal administration of ZD7288 did not. Next, we used La^{3+}, a nonspecific cation channel blocker, to block the membrane depolarization evoked by SNP. The membrane-depolarizing effect of SNP was significantly inhibited by pretreatment with La^{3+} (Figure 7). Recently, it was reported that NO donors could activate nonspecific cation channels including TRPV1 and TRPA1 by direct S-nitrosylation and indirect sGC/PKG pathway [8, 36, 37]. These results indicate that a nonspecific cation channel is involved in NO-related transmission of pain.

5. Conclusion

Substantia gelatinosa neurons in the dorsal horn are critical for mediating nociceptive signals. The dual effect of NO identified in SG neurons is important for the transmission of pain. The findings of this study suggest that NO elicits excitatory and inhibitory effects on SG neurons in a concentration-dependent manner via activation of various ion channels by direct S-nitrosylation and sGC activation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

Acknowledgment

This study was supported by the Wonkwang University in 2011.

References

[1] G. P. Ahern, V. A. Klyachko, and M. B. Jackson, "cGMP and S-nitrosylation: two routes for modulation of neuronal excitability by NO," *Trends in Neurosciences*, vol. 25, no. 10, pp. 510–517, 2002.

[2] V. Calabrese, C. Mancuso, M. Calvani, E. Rizzarelli, D. A. Butterfield, and A. M. Giuffrida Stella, "Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity," *Nature Reviews Neuroscience*, vol. 8, no. 10, pp. 766–775, 2007.

[3] Y. Cury, G. Picolo, V. P. Gutierrez, and S. H. Ferreira, "Pain and analgesia: the dual effect of nitric oxide in the nociceptive system," *Nitric Oxide*, vol. 25, no. 3, pp. 243–254, 2011.

[4] J. V. Esplugues, "NO as a signalling molecule in the nervous system," *British Journal of Pharmacology*, vol. 135, no. 5, pp. 1079–1095, 2002.

[5] N. Olson and A. Van Der Vliet, "Interactions between nitric oxide and hypoxia-inducible factor signaling pathways in inflammatory disease," *Nitric Oxide*, vol. 25, no. 2, pp. 125–137, 2011.

[6] J. W. Denninger and M. A. Marletta, "Guanylate cyclase and the NO/cGMP signaling pathway," *Biochimica et Biophysica Acta*, vol. 1411, no. 2-3, pp. 334–350, 1999.

[7] A. Rudkouskaya, V. Sim, A. A. Shah, P. J. Feustel, D. Jourd'heuil, and A. A. Mongin, "Long-lasting inhibition of presynaptic metabolism and neurotransmitter release by protein S-nitrosylation," *Free Radical Biology & Medicine*, vol. 49, no. 5, pp. 757–769, 2010.

[8] Y. J. Jin, J. Kim, and J. Y. Kwak, "Activation of the cGMP/protein kinase G pathway by nitric oxide can decrease TRPV1 activity in cultured rat dorsal root ganglion neurons," *The Korean Journal of Physiology & Pharmacology*, vol. 16, no. 3, pp. 211–217, 2012.

[9] H. K. Kim, S. K. Park, J.-L. Zhou et al., "Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain," *Pain*, vol. 111, no. 1-2, pp. 116–124, 2004.

[10] H. K. Kim, J. H. Kim, X. Gao et al., "Analgesic effect of vitamin E is mediated by reducing central sensitization in neuropathic pain," *Pain*, vol. 122, no. 1-2, pp. 53–62, 2006.

[11] Y. Guan, M. Yaster, S. N. Raja, and Y.-X. Tao, "Genetic knockout and pharmacologic inhibition of neuronal nitric oxide synthase attenuate nerve injury-induced mechanical hypersensitivity in mice," *Molecular Pain*, vol. 3, article no. 29, 2007.

[12] M. Tanabe, Y. Nagatani, K. Saitoh, K. Takasu, and H. Ono, "Pharmacological assessments of nitric oxide synthase isoforms and downstream diversity of NO signaling in the maintenance of thermal and mechanical hypersensitivity after peripheral nerve injury in mice," *Neuropharmacology*, vol. 56, no. 3, pp. 702–708, 2009.

[13] Y.-C. Chu, Y. Guan, J. Skinner, S. N. Raja, R. A. Johns, and Y.-X. Tao, "Effect of genetic knockout or pharmacologic inhibition of neuronal nitric oxide synthase on complete Freund's adjuvant-induced persistent pain," *Pain*, vol. 119, no. 1–3, pp. 113–123, 2005.

[14] T. J. Coderre and K. Yashpal, "Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model," *The European Journal of Neuroscience*, vol. 6, no. 8, pp. 1328–1334, 1994.

[15] S. T. Meller, C. P. Cummings, R. J. Traub, and G. F. Gebhart, "The role of nitric oxide in the development and maintenance of the hyperalgesia produced by intraplantar injection of carrageenan in the rat," *Neuroscience*, vol. 60, no. 2, pp. 367–374, 1994.

[16] E. Chung, B. Burke, A. J. Bieber, J. C. Doss, Y. Ohgami, and R. M. Quock, "Dynorphin-mediated antinociceptive effects of l-arginine and SIN-1 (an NO donor) in mice," *Brain Research Bulletin*, vol. 70, no. 3, pp. 245–250, 2006.

[17] I. D. G. Duarte, B. B. Lorenzetti, and S. H. Ferreira, "Peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway," *European Journal of Pharmacology*, vol. 186, no. 2-3, pp. 289–293, 1990.

[18] T. Kawano, V. Zoga, M. Kimura et al., "Nitric oxide activates ATP-sensitive potassium channels in mammalian sensory neurons: action by direct S-nitrosylation," *Molecular Pain*, vol. 5, article no. 12, 2009.

[19] V. M. Bolotina, S. Najibi, J. J. Palacino, P. J. Pagano, and R. A. Cohen, "Nitric oxide directly activates calcium-dependent

potassium channels in vascular smooth muscle," *Nature*, vol. 368, no. 6474, pp. 850–853, 1994.

[20] H. Y. Kim, S. J. Kim, J. Kim, S. B. Oh, H. Cho, and S. J. Jung, "Effect of nitric oxide on hyperpolarization-activated current in substantia gelatinosa neurons of rats," *Biochemical and Biophysical Research Communications*, vol. 338, no. 3, pp. 1648–1653, 2005.

[21] Y. W. Yoon, B. Sung, and J. M. Chung, "Nitric oxide mediates behavioral signs of neuropathic pain in an experimental rat model," *NeuroReport*, vol. 9, no. 3, pp. 367–372, 1998.

[22] H. Y. Kim, J. Wang, Y. Lu, J. M. Chung, and K. Chung, "Superoxide signaling in pain is independent of nitric oxide signaling," *NeuroReport*, vol. 20, no. 16, pp. 1424–1428, 2009.

[23] A. Kawabata, S. Manabe, Y. Manabe, and H. Takagi, "Effect of topical administration of L-arginine on formalin-induced nociception in the mouse: a dual role of peripherally formed NO in pain modulation," *British Journal of Pharmacology*, vol. 112, no. 2, pp. 547–550, 1994.

[24] K. Li and W.-X. Qi, "Effects of multiple intrathecal administration of L-arginine with different doses on formalin-induced nociceptive behavioral responses in rats," *Neuroscience Bulletin*, vol. 26, no. 3, pp. 211–218, 2010.

[25] A. M. Sousa and W. A. Prado, "The dual effect of a nitric oxide donor in nociception," *Brain Research*, vol. 897, no. 1-2, pp. 9–19, 2001.

[26] U. Pehl and H. A. Schmid, "Electrophysiological responses of neurons in the rat spinal cord to nitric oxide," *Neuroscience*, vol. 77, no. 2, pp. 563–573, 1997.

[27] Z. Lacza, E. M. Horváth, E. Pankotai et al., "The novel red-fluorescent probe DAR-4M measures reactive nitrogen species rather than NO," *Journal of Pharmacological and Toxicological Methods*, vol. 52, no. 3, pp. 335–340, 2005.

[28] H. Kojima, N. Nakatsubo, K. Kikuchi et al., "Detection and imaging of nitric oxide with novel fluorescent indicators: diaminofluoresceins," *Analytical Chemistry*, vol. 70, no. 13, pp. 2446–2453, 1998.

[29] A. Balcerczyk, M. Soszynski, and G. Bartosz, "On the specificity of 4-amino-5-methylamino-2′,7′-difluorofluorescein as a probe for nitric oxide," *Free Radical Biology & Medicine*, vol. 39, no. 3, pp. 327–335, 2005.

[30] L. Yu, P. E. Gengaro, M. Niederberger, T. J. Burke, and R. W. Schrier, "Nitric oxide: a mediator in rat tubular hypoxia/reoxygenation injury," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 5, pp. 1691–1695, 1994.

[31] L. R. Silveira, L. Pereira-Da-Silva, C. Juel, and Y. Hellsten, "Formation of hydrogen peroxide and nitric oxide in rat skeletal muscle cells during contractions," *Free Radical Biology & Medicine*, vol. 35, no. 5, pp. 455–464, 2003.

[32] C. G. Nichols, "K_{ATP} channels as molecular sensors of cellular metabolism," *Nature*, vol. 440, no. 7083, pp. 470–476, 2006.

[33] O. Behrend, C. Schwark, T. Kunihiro, and M. Strupp, "Cyclic GMP inhibits and shifts the activation curve of the delayed rectifier (IK1) of type I mammalian vestibular hair cells," *NeuroReport*, vol. 8, no. 12, pp. 2687–2690, 1997.

[34] J. Han, N. Kim, E. Kim, W.-K. Ho, and Y. E. Earm, "Modulation of ATP-sensitive potassium channels by cGMP-dependent protein kinase in rabbit ventricular myocytes," *Journal of Biological Chemistry*, vol. 276, no. 25, pp. 22140–22147, 2001.

[35] Q. Sun, G.-G. Xing, H.-Y. Tu, J.-S. Han, and Y. Wan, "Inhibition of hyperpolarization-activated current by ZD7288 suppresses ectopic discharges of injured dorsal root ganglion neurons in a rat model of neuropathic pain," *Brain Research*, vol. 1032, no. 1-2, pp. 63–69, 2005.

[36] T. Miyamoto, A. E. Dublin, M. J. Petrus, and A. Patapoutian, "TRPV1 and TRPA1 mediate peripheral nitric oxide-induced nociception in mice," *PLoS ONE*, vol. 4, no. 10, Article ID e7596, 2009.

[37] T. Yoshida, R. Inoue, T. Morii et al., "Nitric oxide activates TRP channels by cysteine S-nitrosylation," *Nature Chemical Biology*, vol. 2, no. 11, pp. 596–607, 2006.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2013, Article ID 740581, 12 pages
http://dx.doi.org/10.1155/2013/740581

Research Article

Activation of Glycine and Extrasynaptic $GABA_A$ Receptors by Taurine on the Substantia Gelatinosa Neurons of the Trigeminal Subnucleus Caudalis

Thi Thanh Hoang Nguyen, Janardhan Prasad Bhattarai, Soo Joung Park, and Seong Kyu Han

Department of Oral Physiology & Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju 561-756, Republic of Korea

Correspondence should be addressed to Seong Kyu Han; skhan@jbnu.ac.kr

Received 17 September 2013; Revised 5 November 2013; Accepted 12 November 2013

Academic Editor: Dong-ho Youn

The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) has been known for the processing and transmission of orofacial nociceptive information. Taurine, one of the most plentiful free amino-acids in humans, has proved to be involved in pain modulation. In this study, using whole-cell patch clamp technique, we investigated the direct membrane effects of taurine and the action mechanism behind taurine-mediated responses on the SG neurons of the Vc. Taurine showed non-desensitizing and repeatable membrane depolarizations and inward currents which remained in the presence of amino-acid receptors blocking cocktail (AARBC) with tetrodotoxin, indicating that taurine acts directly on the postsynaptic SG neurons. Further, application of taurine at different doses (10 μM to 3 mM) showed a concentration dependent depolarizations and inward currents with the EC_{50} of 84.3 μM and 723 μM, respectively. Taurine-mediated responses were partially blocked by picrotoxin (50 μM) and almost completely blocked by strychnine (2 μM), suggesting that taurine-mediated responses are via glycine receptor (GlyR) activation. In addition, taurine (1 mM) activated extrasynaptic $GABA_A$ receptor ($GABA_A$R)-mediated currents. Taken together, our results indicate that taurine can be a target molecule for orofacial pain modulation through the activation of GlyRs and/or extrasynaptic $GABA_A$Rs on the SG neurons.

1. Introduction

Taurine (2-amino-ethane sulfonic acid) is one of the most plentiful free amino-acids in humans [1, 2]. In the human body, taurine is distributed with high concentration in various tissues that are excitable and/or prone to generate free radicals in retina, white blood cells, platelets, central nervous system (CNS), heart, skeletal muscles, spleen, and liver [3]. In physiological condition, taurine is accumulated in brain cells at concentration of 5–70 mM [4, 5] and is released in high amounts under various pathological conditions such as anoxaemia or ischemia and seizure [6–8]. Since its first discovery in 1827, a number of studies have been done to find out the various physiological functions and the significance of taurine. It has been reported that taurine has various functions including bile acid production [9–12], antiarrhythmic effects [13–15], and oxidant scavenging effects [16]. In central nervous system, taurine has also been reported to modulate calcium homeostasis [17, 18], neuronal excitabilities [19, 20], and excitotoxic cell death [21, 22].

The pain transmission from the orofacial region to the trigeminal subnucleus caudalis (Vc) is responsible by the first-order neurons via small-diameter primary afferents including myelinated Aδ- and unmyelinated C-fibers [23, 24], which innervate in lamina I and in much of lamina II of the Vc [25, 26]. The lamina II called substantia gelatinosa (SG), therefore, is thought to be a key site in the processing of orofacial nociceptive information [27, 28]. The majority of neurons in the SG are local interneurons [29]. A substantial number of these interneurons contain gamma-aminobutyric acid (GABA) and glycine which are often colocalized in the same cell [30, 31]. As one of the main inhibitory neurotransmitters

in the central nervous system, GABA and glycine have pivotal roles in the modulation of nociception [32–35].

A number of studies have shown that taurine is involved in pain modulation. For example, systemic and intrathecal administration of taurine induced the antinociceptive effects to inhibit the intensity of caudally-directed biting, scratching, and paw licking behaviors by chemical agent and by the hot-plate test at acute pain tests in mouse [36, 37]. It has been reported that dietary supplementation with taurine suppresses hyperalgesia in streptozotocin-induced diabetic rats and autotomy behavior in genetically selected Sabra strain rats [38]. In addition, Lee et al. showed that taurine is released from neurons in the upper dorsal horn layers which are known to conduct nociceptive input [39]. These previous reports have strongly suggested that taurine can modulate nociceptive information. Similarly, Bereiter et al. reported that there was an elevation of taurine after mustard oil (a chemical irritant) injection through the skin into the temporomandibular joint region in rats [40]. However, the action mechanism of taurine on the SG neurons which are involved in orofacial pain modulation has not been fully understood. In this study, therefore, we used the whole-cell patch clamp technique to investigate the action mechanism of taurine on the SG neurons of the Vc.

2. Materials and Methods

2.1. Animals. All experiments on living animals were ratified by Chonbuk University Animal Welfare and Ethics Committee. Immature male and female ICR mice used in the present study were housed under 12-h light : 12-h dark cycles (lights on at 07:00 h) with access to food and water *ad libitum*.

2.2. Brain Slice Preparation. Brain slice preparation was similar to the work done by Park et al. [41]. Briefly, the juvenile ICR mice (5-20 postnatal days) were decapitated and their brains were excised quickly, immersed in ice-cold bicarbonate-buffered artificial cerebrospinal fluid (ACSF) with the following chemical composition (in mM): 126 NaCl, 2.5 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 11 D-glucose, 1.4 NaH_2PO_4, and 25 $NaHCO_3$ (pH 7.3~7.4, bubbled with 95% O_2 and 5% CO_2). The trigeminal subnucleus caudalis segment was dissected, supported with a 4% agar block, and glued with cyanoacrylate to the chilled stage of a vibratome (Microm, Walldorf, Germany). Coronal slices (150 μm in thickness, obtained 1-2 mm from the obex, the most rostral part of Vc) were prepared in ice-cold ACSF using the vibratome. The slices were kept in oxygenated ACSF at room temperature for at least 1 h before electrophysiological recording.

2.3. Electrophysiological Procedures and Data Analysis. The slices were transferred into a recording chamber, completely submerged, and continuously superfused with carboxygenated ACSF at a rate of 4-5 mL/min. The slices were viewed with an upright microscope (BX51W1, Olympus, Tokyo, Japan) with Nomarski differential interference contrast optics. The SG (lamina II) was clearly identified as a translucent band, just medial to the spinal trigeminal tract and traveled along the lateral edge of the slice. The patch pipettes were pulled from thin-wall borosilicate glass-capillary tubing (PG52154-4, WPI, Sarasota, USA) on a Flaming/Brown, puller (P-97, Sutter Instruments Co., Novato, CA). The pipette solution was passed through a disposable 0.22 μm filter and contained the following composition (in mM): 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 MgATP, and 10 EGTA (pH 7.3 with KOH). In this study, we used high chloride pipette solution to amplify the chloride mediated conductance. The resistance between the recording electrode filled with pipette solution and the reference electrode was 4–6 MΩ. After a gigaohm seal was formed with SG neuron, the cell membrane patch was ruptured by negative pressure, and then the whole-cell patch clamp recording was performed using an Axopatch 200B (Axon Instruments, Union City, CA). The changes in membrane potentials and membrane currents were sampled online using a Digidata 1322A interface (Axon Instruments) connected to a desktop PC. The signals were filtered (2 kHz, Bessel Filter of Axopatch 200B) before digitizing at a rate of 1 kHz. The holding current was not adjusted during the experiment and was set at 0 pA in current clamp mode. The root mean square (RMS) noises were measured in 50 ms epochs of traces lacking postsynaptic currents (PSCs), in periods of control ACSF and in the presence of strychnine and strychnine + taurine 100 μM (n = 50 epochs in each case). The mean holding current changes within the control and treated period were calculated as the mean of peak-to-peak amplitude of individual points within each period. The acquisition and subsequent analysis of the acquired data were performed using Clampex9 software (Axon Instruments, USA). The traces were plotted using Origin7 software (MicroCal Software, Northampton, USA). All recordings were made at room temperature.

2.4. Drugs. The drugs used in the present study were taurine, strychnine, gabazine, picrotoxin, bicuculline (purchased from Sigma, USA), and tetrodotoxin (TTX) (from Tocris, UK). Stocks of all drugs were made according to their solubility in DMSO and in distilled water. Stocks were diluted (usually 1,000 times) to the desired final concentrations in ACSF immediately before use and were applied by bath application (4 mL/min).

2.5. Statistics. All values were expressed as the mean ± S.E.M. A paired t-test and one way ANOVA test were used to examine the difference. Statistical significance was defined as $P < 0.05$.

3. Results

Whole cell current and voltage clamp recordings were obtained from 98 SG neurons from juvenile mice postnatal day ranging from day 5 to day 20. A series of experiments were designed to evaluate the effects of taurine on SG neurons. The mean resting membrane potential of SG neurons tested in current clamp mode was −59.4 ± 1.61 mV (n = 25).

3.1. Taurine Induces Nondesensitizing Membrane Potential and Holding Current Changes on SG Neuron. In current

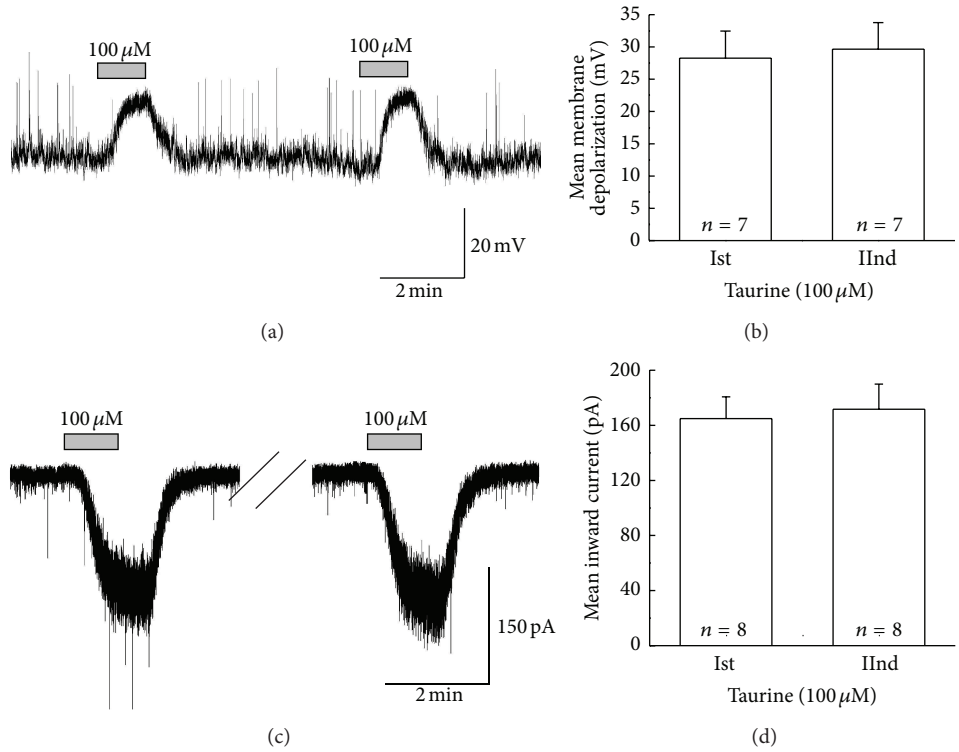


Figure 1: Repeated responses by the successive application of taurine on SG neurons. (a), (c) The representative traces show the repeatable membrane depolarization and repeated inward current induced by taurine (100 μM). (b), (d) Bar graphs illustrate the comparison of the mean membrane potential and inward current changes by the repeated application of taurine (100 μM) ($P > 0.05$).

and voltage clamp mode, taurine (100 μM) was applied repeatedly at 5-minute time intervals to determine if the SG neurons were desensitized by successive application. In 7 SG neurons tested in current clamp mode, taurine (100 μM) induced repeated membrane depolarizations (Figure 1(a)). When taurine was successively applied, the mean membrane potential change (29.7 ± 4.12 mV) by the second application was similar to that of the first application (28.3 ± 4.20 mV, $n = 7, P > 0.05$, Figure 1(b)). Similarly, in voltage clamp mode at holding potential of −60 mV, taurine (100 μM) induced repeated inward currents (Figure 1(c)). When taurine was successively applied, the mean inward current (−172 ± 18.3 pA) by the second application was similar to that of the first application (−165±15.9 pA, $n = 8, P > 0.05$, Figure 1(d)). These results indicate that SG neurons are not desensitized by the successively applied taurine that induces inhibitory depolarizing potentials or inward currents, respectively, at current clamp or voltage clamp mode. The mean relative membrane depolarization and the mean relative inward current of the second application were 1.06 ± 0.03 ($n = 7$) and 1.03 ± 0.04 ($n = 8$), respectively.

3.2. Postsynaptic Action of Taurine on SG Neurons. To investigate whether taurine affects SG neuronal activities via action potential mediated presynaptic release, the effects of taurine were examined in the presence of tetrodotoxin (TTX), a voltage sensitive Na^+ channel blocker in current and voltage clamp mode. Taurine (100 μM) induced membrane depolarization and when TTX (0.5 μM) was applied, spontaneous action potentials were rapidly abolished. However, TTX did not affect the taurine-induced membrane depolarization. The mean membrane potential change (26.7 ± 4.60 mV, $n = 7$) in the presence of TTX 0.5 μM was similar to that of taurine alone (28.4 ± 3.91 mV, $n = 7$, $P > 0.05$). Further, in voltage clamp experiment, the taurine-mediated inward current was not blocked by TTX. The mean inward current change (155 ± 54.6 pA, $n = 3$) in the presence of TTX was similar to that of taurine alone (162 ± 80.5 mV, $n = 7$, $P > 0.05$) (figure not shown). These results indicate that taurine-induced responses were not mediated via any action potential dependent presynaptic action on the SG neurons.

Further, we used amino-acid receptors blocking cocktail (AARBC) (6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) 10 μM and (2R)-amino-5-phosphonovaleric acid (AP5) 20 μM, gabazine 3 μM along with tetrodotoxin (TTX) 0.5 μM) to find out if taurine affects SG neuronal activities directly on the postsynaptic site. As shown in Figures 2(a) and 2(c), there were no significant differences between the responses induced by taurine alone and in the presence of AARBC. The amplitude of mean membrane depolarization induced by taurine alone (17.8 ± 4.16 mV, $n = 4$) was nearly similar to that of in the presence of AARBC (20.8 ± 4.09 mV, $n = 4$, $P > 0.05$, Figure 2(b)). Similarly, taurine-evoked mean inward currents in taurine alone and in the presence of AARBC were also almost equal (109 ± 33.4 pA and

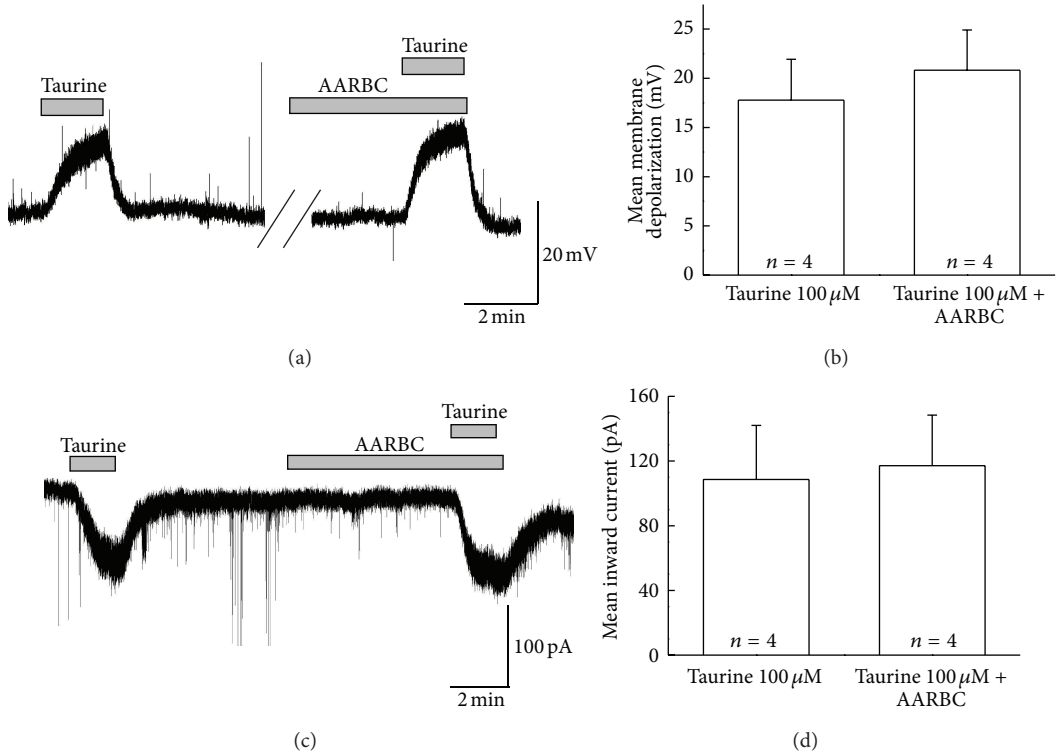


Figure 2: Taurine-induced membrane depolarizations and taurine-induced currents are mediated by postsynaptic SG neurons. (a), (c) The representative traces showing membrane depolarization and inward current induced by taurine (100 μM) alone and taurine in the presence of AARBC. (b), (d) Bar graphs showing the comparisons of mean relative membrane depolarization and mean inward current by the taurine alone and taurine in the presence of AARBC ($P > 0.05$).

117 ± 31.3 pA, resp., $n = 4$, $P > 0.05$, Figure 2(d)). These results put forth that taurine-mediated inward currents and depolarizations were purely postsynaptic events.

Taurine-induced membrane depolarizations and inward currents were examined at different concentrations ranging from 10 to 3,000 μM. Figures 3(a) and 3(c) show the representative traces indicating the clear concentration dependency by taurine applications. Taurine-induced membrane depolarizations and inward currents were bigger at higher concentrations. Figure 3(b) illustrates the mean membrane depolarization changes by taurine at different concentrations (10 μM: 0.38 ± 0.15 mV, 30 μM: 5.74 ± 2.33 mV, 100 μM: 16.1 ± 4.95 mV, 300 μM: 26.9 ± 4.03 mV, 1,000 μM: 30.3 ± 4.80 mV, $n = 7$) with an EC_{50} of 84.3 μM. Similarly, there was an increase of mean inward currents following the rise of concentration in voltage clamp mode as well (10 μM: 2.88 ± 0.81 pA, 30 μM: 7.06 ± 2.46 pA, 100 μM: 43.9 ± 5.27 pA, 300 μM: 192 ± 29.9 pA, 1,000 μM: 583 ± 138 pA, 3,000 μM: 842 ± 155 pA, $n = 8$) with an EC_{50} of 723 μM. The values of EC_{50} were estimated by curve fitting using Origin software. This discrepancy of EC_{50} values between voltage and current clamp may be explained due to the activation of certain voltage-sensitive ion channels in current clamp mode. These concentration dependent responses also support that taurine acts on the postsynaptic site of SG neurons directly.

3.3. Taurine Activates Glycine Receptors on SG Neurons. It has been reported that taurine can activate GlyRs in ventromedial hypothalamic neurons [42], supraoptic magnocellular neurons [43], cultured neurons of auditory cortex [44], and anteroventral cochlear nucleus neurons [45]. To check whether taurine-induced membrane depolarizations and inward currents on the SG neurons of the Vc were mediated by GlyR activation, strychnine, a selective GlyR antagonist was used. As shown in Figures 4(a) and 4(c), taurine-induced membrane depolarization and current were almost blocked by strychnine (2 μM). The mean membrane depolarizations induced by the application of taurine in the absence and presence of strychnine were 28.5 ± 5.14 mV and 1.25 ± 0.19 mV, respectively ($n = 6$, Figure 4(b), $P < 0.01$). In addition, the mean inward current induced by taurine (205 ± 57.4 pA) was eliminated by the simultaneous application with strychnine (1.38 ± 0.58 pA) ($n = 7$, Figure 4(d), $P < 0.05$).

3.4. Taurine-Induced Actions Were Mediated via GlyRs and Extrasynaptic $GABA_A$ Receptors. It has been reported that taurine can activate $GABA_A$ receptors ($GABA_A$Rs) in various regions such as main olfactory bulb [46, 47], in the hippocampal CA1 area [48], and in anteroventral cochlear nucleus neurons [45]. As gabazine is well known to block synaptic $GABA_A$Rs at lower concentration [49] as well as extrasynaptic $GABA_A$Rs at higher concentration [50], taurine was applied in the presence of gabazine (3 μM).

The currents activated by taurine at 100 μM and 1,000 μM were not affected by 3 μM gabazine (Figures 5(a) and 5(c)). Figures 5(b) and 5(d) compare the changes in inward currents

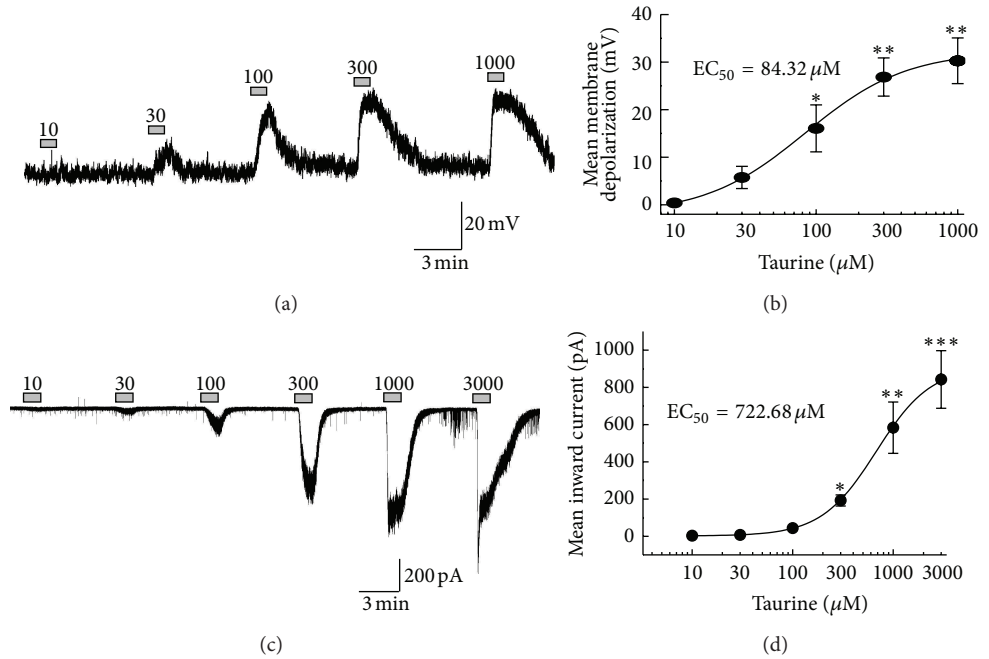


Figure 3: Concentration-response relationship. (a), (c) Representative traces of SG neurons showing the changes of membrane depolarizations and inward currents to different doses of taurine (10, 30, 100, 300, 1,000, 3,000 μM). (b), (d) Curve figures showing the mean membrane potentials and the mean inward currents change which correspond with the concentration changes ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, one-way ANOVA, Scheffe's post hoc test).

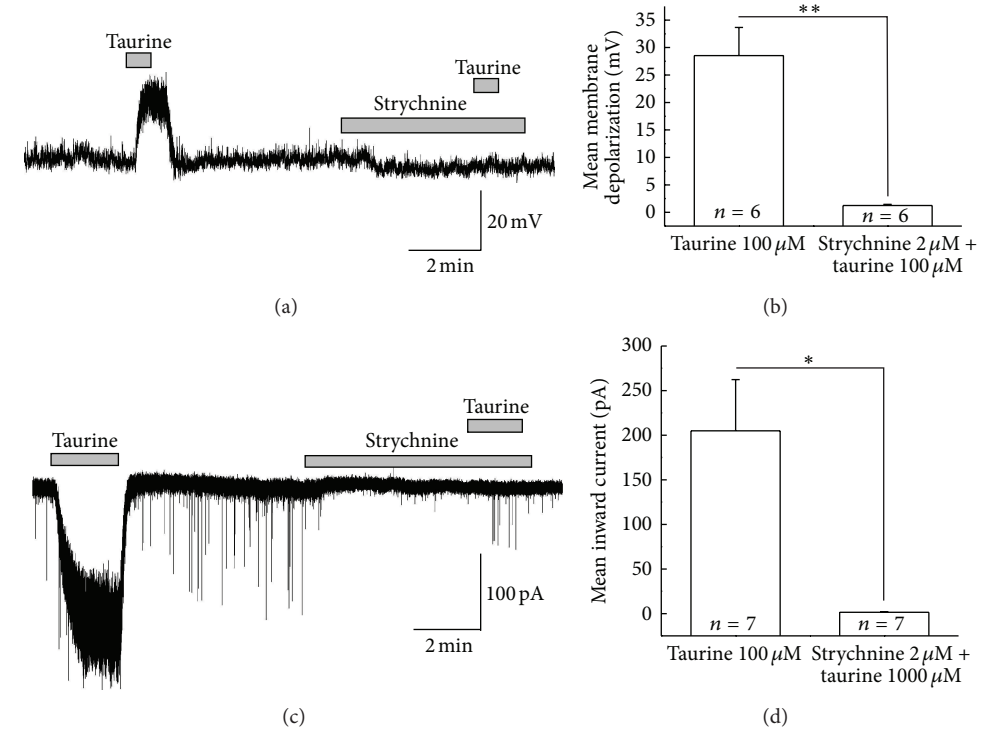


Figure 4: Inhibition of taurine-induced membrane depolarization and inward current by strychnine on SG neurons of Vc. (a), (c) Representative traces showing the taurine-induced membrane depolarization and taurine-induced inward current were blocked by strychnine (2 μM), a glycine receptor (GlyR) antagonist. (b), (d) Bar graphs showing the comparisons of mean relative membrane potential and inward current changed by the taurine alone and in the presence of strychnine ($^{*}P < 0.05$, $^{**}P < 0.01$).

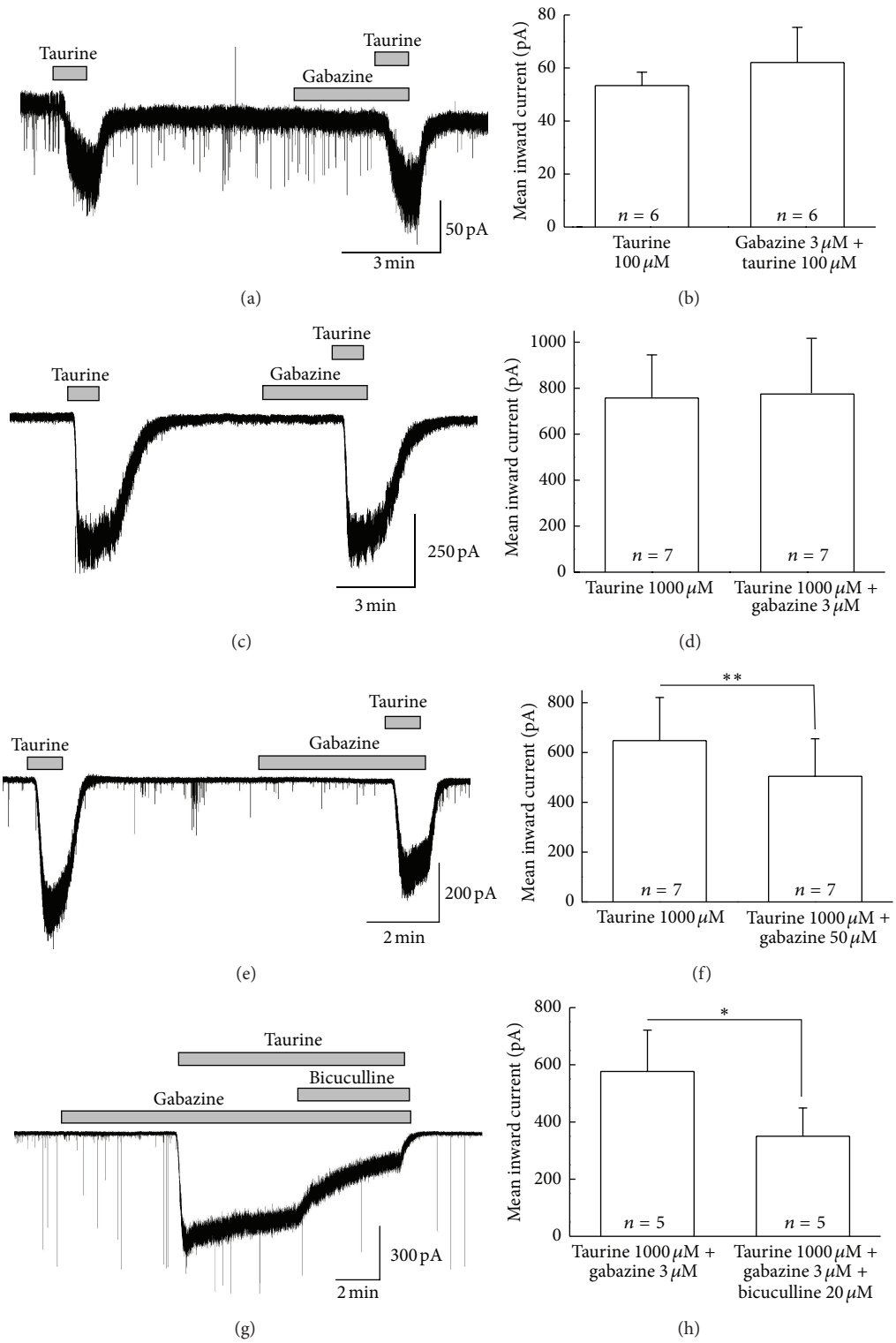


Figure 5: Taurine-induced inward current is only sensitive to gabazine at high concentration on SG neurons. (a), (c), (e) The representative traces showing the responses to taurine (100 μM and 1,000 μM) were not affected by gabazine 3 μM but were affected by gabazine 50 μM. (b), (d), (f) Bar graphs showing no significant difference about mean inward currents between the application taurine alone and taurine in the presence of gabazine 3 μM ($P > 0.05$), but there was a considerable change in the presence of gabazine 50 μM ($P < 0.01$). (g) The representative trace showing the inhibition of taurine-induced inward current in the presence of gabazine by $GABA_A$ broad antagonist bicuculline (20 μM). (h) The bar graph showing the mean inward current induced by taurine 1,000 μM in the presence of gabazine 3 μM and the mean remaining response after being blocked by bicuculline 20 μM ($P < 0.05$). Holding potential was −60 mV.

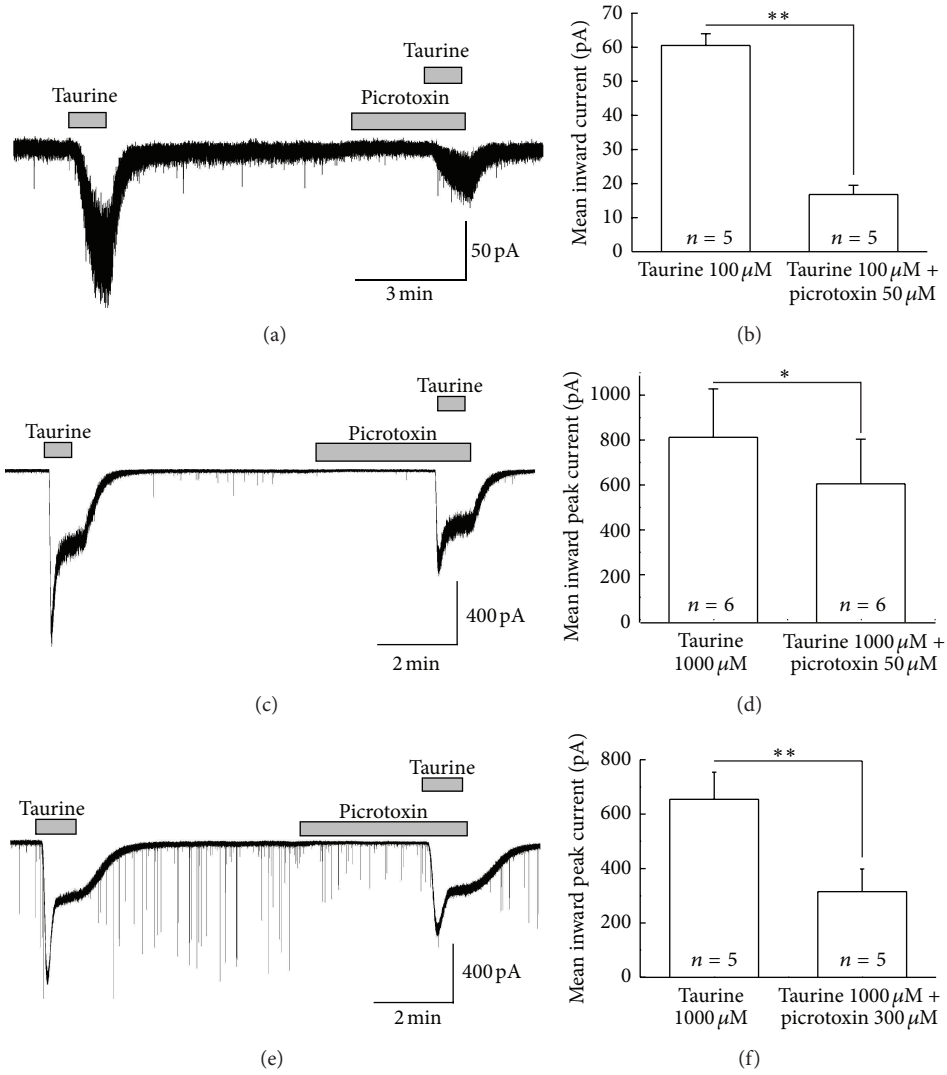


FIGURE 6: Taurine-induced inward current is sensitive to picrotoxin on SG neurons. (a), (c), (e) The representative traces showing currents evoked by 100 μM and 1,000 μM taurine were blocked by picrotoxin 50 μM and 300 μM. (b), (d), (f) Comparison of mean inward current changed by taurine alone with taurine in the presence of picrotoxin ($^{*}P < 0.05$, $^{**}P < 0.01$). Holding potential was −60 mV.

between taurine alone (with two different concentrations 100 μM and 1,000 μM (53.4 ± 5.06 pA and 758 ± 187 pA, resp.)) and taurine in the presence of gabazine 3 μM (62.1 ± 13.3 pA and 774 ± 235 pA, resp.). Therefore, at these concentrations, $GABA_A$Rs are not affected by taurine. On the other hand, to identify whether taurine can act on extrasynaptic $GABA_A$Rs on SG neurons, the concentration of gabazine was increased to 50 μM (Figures 5(e) and 5(f)). The taurine-induced current was inhibited by gabazine at high concentration (Figure 5(e)). Specifically, the mean inward current induced by taurine 1,000 μM (648 ± 173 pA) was reduced to 504 ± 151 pA in the presence of gabazine 50 μM (Figure 5(f), $P < 0.01$). Further additional experiments in the presence of gabazine and bicuculline were conducted to figure out the activation of extrasynaptic $GABA_A$Rs current by 1,000 μM taurine, and as expected, bicuculline blocked the taurine-induced inward current in the presence of gabazine (Figures 5(g) and 5(h), $P < 0.05$).

There are a plethora of studies suggesting that the $GABA_A$R receptor antagonist picrotoxin also blocks extrasynaptic homomeric glycine receptors at lower concentration of 50–100 μM and is used extensively to characterize the glycine receptors on neuronal populations. So, here in this study we tested taurine in the presence of picrotoxin to characterize the type GlyRs activated by taurine on SG neurons of Vc. Taurine-induced inward currents on SG neurons were blocked by picrotoxin 50 μM (Figures 6(a) and 6(c)). The mean inward currents evoked by taurine 100 μM and 1,000 μM were significantly decreased in the presence of picrotoxin (50 μM). The mean inward currents evoked by taurine 100 μM and 1,000 μM in absence and presence of picrotoxin were 60.5 ± 3.43 pA; 813 ± 216 pA and 16.8 ± 2.71 pA; and 605 ± 199 pA, respectively (Figures 6(b) and 6(d)). These results suggest that the SG neurons of Vc functionally express both heteromeric and homomeric GlyRs. Interestingly, it is very clear from Figures 6(b) and 6(d) that the inhibition of

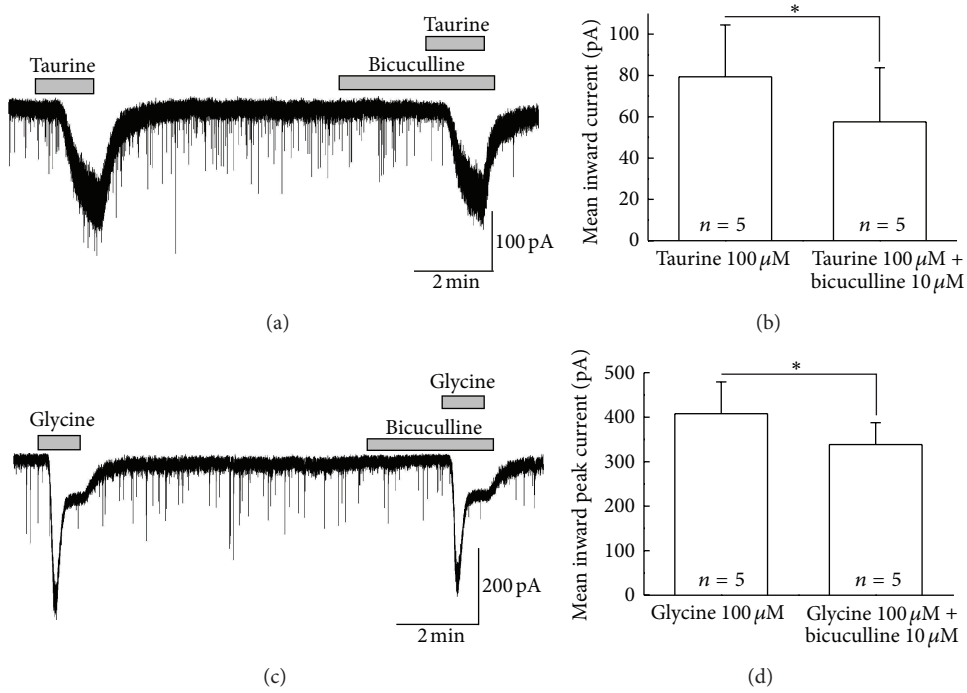


Figure 7: Sensitivity of taurine- and glycine-induced current to bicuculline. (a), (c) Currents activated by taurine and glycine were inhibited by bicuculline. (b), (d) The bar graphs show that mean inward currents effected by taurine and glycine were both reduced by the simultaneous application of bicuculline ($P < 0.05$) Holding potential was −60 mV.

1,000 μM taurine-mediated response by picrotoxin (50 μM) was less than that of 100 μM taurine. This result can be explained considering that there might be a possibility that at higher concentration of taurine may affect extrasynaptic $GABA_A$Rs. In addition, at high concentration of picrotoxin (300 μM), 1,000 μM taurine-induced currents were further decreased (Figures 6(e) and 6(f)), suggesting the activation of extrasynaptic $GABA_A$Rs by higher concentration of taurine.

Following this further, we also used another selective $GABA_A$R antagonist, bicuculline, which follows the same pattern as picrotoxin does, that is, blockade of homomeric GlyRs [51]. We confirmed the inhibitory effect of bicuculline on taurine and glycine-mediated responses. Figures 7(a) and 7(c) show the inhibition of bicuculline on the taurine and glycine-induced currents. The mean inward currents by taurine 100 μM in the absence and presence of bicuculline 10 μM were 79.3 ± 25.1 pA and 57.6 ± 26.2 pA (Figure 7(b)), respectively. Whereas the mean inward currents elicited by glycine (100 μM) in the absence and presence of bicuculline (10 μM) were 408 ± 71.5 pA and 339 ± 48.6 pA (Figure 7(d), $n = 5$), respectively.

Further, in a quest to figure out the actual extrasynaptic glycine and $GABA_A$ receptors mediated tonic currents by 1,000 μM taurine on SG neurons, it was applied in the presence of strychnine. Strychnine dramatically blocked the synaptic currents and induced outward shift of the holding current (Figure 8(a)). Presumably, this blockade of synaptic currents were via heteromeric GlyRs, and outward shift of holding current was induced via extrasynaptic GlyRs. Moreover in the presence of strychnine, taurine (1,000 μM) induced the inward current with increase in RMS noise. RMS noise in intact condition, in the presence of strychnine and in the presence of strychnine and taurine were 3.45 ± 0.28 pA, 2.23 ± 0.18 pA and 3.56 ± 0.23 pA, respectively ($n = 7$, Figure 8(b), $P < 0.01$).

4. Discussion

The results of this study can be summarized as follows. SG neurons were not desensitized by the application of taurine. The taurine-induced membrane depolarizations on SG neurons were mediated by postsynaptic actions. There was concentration-response relationship between taurine and SG neurons. Taurine acted as an agonist on both extrasynaptic homomeric and synaptic hetromeric GlyRs on the SG neurons. Taurine at higher concentration could affect extrasynaptic $GABA_A$Rs.

Taurine has been demonstrated for its ability in modulation of synaptic transmission by activating GlyRs and/or $GABA_A$Rs. However, the physiological actions of taurine which can be upon either GlyRs or $GABA_A$Rs have been also proved to depend on the specific brain region studied [46, 47]. For example, taurine activates both $GABA_A$Rs and GlyRs in neurons of the supraoptic nucleus, Xenopus oocytes, and the hippocampal CA1 area [43, 48, 52] and activates only $GABA_A$Rs receptors in mitral and tufted cells from the rat main olfactory bulb [47]. In addition, this activation of taurine in some brain regions is concentration-dependent. For instance, in young rat hippocampus, nucleus accumbens, and adult rat supraopic nucleus, taurine cannot only activate GlyRs at a low concentration (≤1 mM) but can activate $GABA_A$Rs as well at a high concentration (≥3 mM)

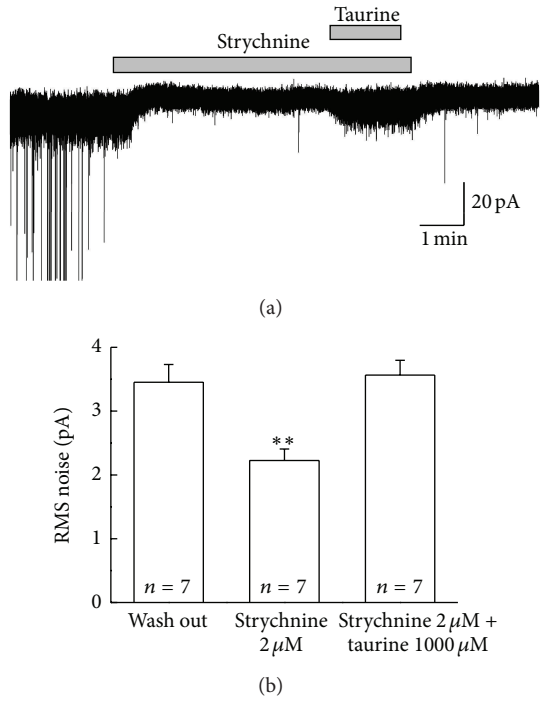


Figure 8: Taurine-mediated tonic conductance via extrasynaptic glycine and GABA receptors on SG neurons. (a) The representative trace illustrated that strychnine 2 μM mediated an outward shift of holding current by blocking glycine-mediated neurotransmission and blocked the taurine-induced synaptic currents except $GABA_A$R-mediated extrasynaptic current. (b) The bar graph showing the comparison of RMS noise in intact condition, in the presence of strychnine 2 μM and in the spontaneous application of taurine 1,000 μM and strychnine 2 μM ($^{**}P < 0.01$). Holding potential was −60 mV.

[43, 48, 53]. On the other hand, the findings by Song et al. in 2012 have shown that in anteroventral cochlear nucleus neurons, at low (0.1 mM) and high (1 mM) concentrations, taurine can activate both $GABA_A$Rs and GlyRs [45].

In the mammalian CNS, GlyRs are formed by a combination of five distinct transmembrane protein subunits, one β subunit and four α subunit (α1–α4) [54, 55]. This composition influences in two different ways of forming functional receptors: the homomeric configuration comprising five α subunits and the heteromeric configuration composed of $2\alpha : 3\beta$ subunits [55–57]. The physiological and pharmacological properties of GlyRs are dependent on the subunit combination. Picrotoxin, a $GABA_A$R antagonist, is proved as a standard tool to discriminate between homomeric and heteromeric GlyRs [58]. At low concentration of 50–100 μM, picrotoxin selectively blocks homomeric GlyRs but not heteromeric receptors. In this study, to pharmacologically characterize the type of GlyRs present on SG neurons, taurine and glycine 100 μM were applied in the presence of picrotoxin. The result indicate that glycine- and taurine-induced inward currents were partially blocked by picrotoxin (50 μM), suggesting the presence of α homomeric GlyRs. However, this blockade was not complete and the unblocked remainder implies the activation of another GlyRs, likely $\alpha\beta$ heteromeric GlyRs. The result in this study puts forth that taurine activates not only the synaptic hetromeric GlyRs but also the homomeric extrasynaptic GlyRs giving the tonic glycinergic inhibition on SG neurons, as established on spinal cord and hippocampal neurons [59, 60].

Another major inhibitory neurotransmitter in the CNS is GABA which mediates its most rapid effects via the ionotropic $GABA_A$Rs. $GABA_A$Rs which are pentameric ligand-gated ion channels consisting of diverse subunits are typically composed of two α and two β subunits together with γ2 subunit [61]. The difference of subunit composition influences not only the properties and function of receptors but also their distribution within the cellular membrane [62, 63]. $GABA_A$ receptors, containing the γ2 subunit, are preferentially located in the synapse and generate "phasic" inhibitory postsynaptic currents [64]. On the other hand, in some receptors, the δ subunit can take the place of the γ2 subunit. The existence of the δ subunit leads to receptor expression in the extrasynaptic membrane and the activation of these receptor results in the generation of "tonically" active currents [65–68]. In the present study, inward current with increased RMS noise by taurine 1,000 μM in the presence of strychnine and unaffected current in the presence of gabazine 3 μM which blocks the synaptic $GABA_A$Rs suggests the activation of extrasynaptic $GABA_A$Rs by taurine 1,000 μM. The activation of extrasynaptic $GABA_A$Rs by taurine may have important physiological and pathophysiological effects to protect neurons from toxicity under pathological conditions [22].

Glycine and GABA are known to be inhibitory neurotransmitters. Within the SG of the spinal dorsal horn, these neurotransmitters take part in the modulation of sensory input by exerting powerful inhibitory effects on spontaneous and afferent evoked activity in second-order neurons [69]. In previous studies, $GABA_A$R- and GlyR-mediated conductance have been found to have inhibitory effects on orofacial nociceptive input [70]. Likewise taurine has also been shown to have inhibitory effect on other brain areas [71]. In this study, activation of glycine and GABA receptors by taurine on SG neurons has given a clear evidence that taurine behaves as an inhibitory neurotransmitter on the SG neurons of Vc. Because of this property, taurine symbolizes essential targets in descending pathways to orofacial pain.

The significant increase of taurine level in the brain under pathological conditions in response to electrical, chemical, and pain stimulation signals that taurine may play a role in neuroprotection [72–74]. With the physiological ability to activate the inhibitory neurotransmitter receptor in SG neurons, our results indicate that the influence of taurine on SG neurons may be an important modulation which has a part in the processing of orofacial nociceptive information. Further researches need to be done to ascertain the antinociceptive role of taurine to orofacial pain.

Acknowledgment

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF)

funded by the Ministry of Education, Science and Technology (2012R1A1A2003535).

References

[1] M. Neuringer and J. Sturman, "Visual acuity loss in rhesus monkey infants fed a taurine-free human infant formula," *The Journal of Neuroscience Research*, vol. 18, no. 4, pp. 597–601, 1987.

[2] M. Neuringer, H. Imaki, J. A. Sturman, R. Moretz, and H. M. Wisniewski, "Abnormal visual acuity and retinal morphology in rhesus monkeys fed a taurine-free diet during the first three postnatal months," *Advances in Experimental Medicine and Biology*, vol. 217, pp. 125–134, 1987.

[3] J. G. Jacobsen and L. H. Smith, "Biochemistry and physiology of taurine and taurine derivatives," *Physiological Reviews*, vol. 48, no. 2, pp. 424–511, 1968.

[4] H. Benrabh, J.-M. Bourre, and J.-M. Lefauconnier, "Taurine transport at the blood-brain barrier: an in vivo brain perfusion study," *Brain Research*, vol. 692, no. 1-2, pp. 57–65, 1995.

[5] N. Del Olmo, M. Galarreta, J. Bustamante, R. Martín Del Río, and J. M. Solís, "Taurine-induced synaptic potentiation: role of calcium and interaction with LTP," *Neuropharmacology*, vol. 39, no. 1, pp. 40–54, 2000.

[6] P. Saransaari and S. S. Oja, "Modulation of the ischemia-induced taurine release by adenosine receptors in the developing and adult mouse hippocampus," *Neuroscience*, vol. 97, no. 3, pp. 425–430, 2000.

[7] J. W. Phillis and M. H. O'Regan, "Characterization of modes of release of amino acids in the ischemic/reperfused rat cerebral cortex," *Neurochemistry International*, vol. 43, no. 4-5, pp. 461–467, 2003.

[8] Z. Li, X. Zhang, X. Lu, M. Zhong, and Y. Ji, "Dynamic release of amino acid transmitters induced by valproate in PTZ-kindled epileptic rat hippocampus," *Neurochemistry International*, vol. 44, no. 4, pp. 263–270, 2004.

[9] F. Guertin, C. C. Roy, G. Lepage et al., "Effect of taurine on total parenteral nutrition-associated cholestasis," *Journal of Parenteral and Enteral Nutrition*, vol. 15, no. 3, pp. 247–251, 1991.

[10] P. Invernizzi, K. D. R. Setchell, A. Crosignani et al., "Differences in the metabolism and disposition of ursodeoxycholic acid and of its taurine-conjugated species in patients with primary biliary cirrhosis," *Hepatology*, vol. 29, no. 2, pp. 320–327, 1999.

[11] S. Caglieris, E. Giannini, G. Dardano, L. Mondello, U. Valente, and R. Testa, "Tauroursodeoxycholic acid administration as adjuvant therapy in cirrhotic patients on transplantation waiting lists," *Hepato-Gastroenterology*, vol. 47, no. 34, pp. 1045–1047, 2000.

[12] Y. Sunami, S. Tazuma, and G. Kajiyama, "Gallbladder dysfunction enhances physical density but not biochemical metastability of biliary vesicles," *Digestive Diseases and Sciences*, vol. 45, no. 12, pp. 2382–2391, 2000.

[13] H. Satoh and N. Sperelakis, "Review of some actions of taurine on ion channels of cardiac muscle cells and others," *General Pharmacology*, vol. 30, no. 4, pp. 451–463, 1998.

[14] L. Niittynen, M. Nurminen, R. Korpela, and H. Vapaatalo, "Role of arginine, taurine and homocysteine in cardiovascular diseases," *Annals of Medicine*, vol. 31, no. 5, pp. 318–326, 1999.

[15] M. J. Sole and K. N. Jeejeebhoy, "Conditioned nutritional requirements and the pathogenesis and treatment of myocardial failure," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 3, no. 6, pp. 417–424, 2000.

[16] C. Cunningham, K. F. Tipton, and H. B. F. Dixon, "Conversion of taurine into N-chlorotaurine (taurine chloramine) and sulphoacetaldehyde in response to oxidative stress," *Biochemical Journal*, vol. 330, no. 2, pp. 939–945, 1998.

[17] H. Pasantes-Morales and A. Gamboa, "Effect of taurine on $^{45}Ca^{2+}$ accumulation in rat brain synaptosomes," *Journal of Neurochemistry*, vol. 34, no. 1, pp. 244–246, 1980.

[18] W. Q. Chen, H. Jin, M. Nguyen et al., "Role of taurine in regulation of intracellular calcium level and neuroprotective function in cultured neurons," *The Journal of Neuroscience Research*, vol. 66, no. 4, pp. 612–619, 2001.

[19] F. Wang, C. Xiao, and J. H. Ye, "Taurine activates excitatory non-synaptic glycine receptors on dopamine neurones in ventral tegmental area of young rats," *Journal of Physiology*, vol. 565, no. 2, pp. 503–516, 2005.

[20] H. Xu, W. Wang, Z. Tang, T. Xu, and L. Chen, "Taurine acts as a glycine receptor agonist in slices of rat inferior colliculus," *Hearing Research*, vol. 220, no. 1-2, pp. 95–105, 2006.

[21] P. Saransaari and S. S. Oja, "Taurine and neural cell damage," *Amino Acids*, vol. 19, no. 3-4, pp. 509–526, 2000.

[22] P. R. Louzada, A. C. P. Lima, D. L. Mendonça-Silva, F. Noël, F. G. De Mello, and S. T. Ferreira, "Taurine prevents the neurotoxicity of β-amyloid and glutamate receptor agonists: activation of GABA receptors and possible implications for Alzheimer's disease and other neurological disorders," *The FASEB Journal*, vol. 18, no. 3, pp. 511–518, 2004.

[23] A. R. Light and E. R. Perl, "Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers," *Journal of Comparative Neurology*, vol. 186, no. 2, pp. 133–150, 1979.

[24] Y. Sugiura, C. L. Lee, and E. R. Perl, "Central projections of identified, unmyelinated (C) afferent fibers innervating mammalian skin," *Science*, vol. 234, no. 4774, pp. 358–361, 1986.

[25] W. D. Willis and K. N. Westlund, "Neuroanatomy of the pain system and of the pathways that modulate pain," *Journal of Clinical Neurophysiology*, vol. 14, no. 1, pp. 2–31, 1997.

[26] L. Lorenzo, M. Ramien, M. St. Louis, Y. De Koninck, and A. Ribeiro-da-Silva, "Postnatal changes in the rexed lamination and markers of nociceptive afferents in the superficial dorsal horn of the rat," *Journal of Comparative Neurology*, vol. 508, no. 4, pp. 592–604, 2008.

[27] B. J. Sessle, "Acute and chronic craniofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates," *Critical Reviews in Oral Biology and Medicine*, vol. 11, no. 1, pp. 57–91, 2000.

[28] S. F. A. Santos, S. Rebelo, V. A. Derkach, and B. V. Safronov, "Excitatory interneurons dominate sensory processing in the spinal substantia gelatinosa of rat," *Journal of Physiology*, vol. 581, no. 1, pp. 241–254, 2007.

[29] J. M. Braz, M. A. Nassar, J. N. Wood, and A. I. Basbaum, "Parallel "pain" pathways arise from subpopulations of primary afferent nociceptor," *Neuron*, vol. 47, no. 6, pp. 787–793, 2005.

[30] A. J. Todd and A. C. Sullivan, "Light microscope study of the coexistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat," *Journal of Comparative Neurology*, vol. 296, no. 3, pp. 496–505, 1990.

[31] A. J. Todd, C. Watt, R. C. Spike, and W. Sieghart, "Colocalization of GABA, glycine, and their receptors at synapses in the rat spinal cord," *The Journal of Neuroscience*, vol. 16, no. 3, pp. 974–982, 1996.

[32] R. L. Macdonald and R. W. Olsen, "GABA(A) receptor channels," *Annual Review of Neuroscience*, vol. 17, pp. 569–602, 1994.

[33] T. J. Price, F. Cervero, and Y. de Koninck, "Role of cation-chloride-cotransporters (CCC) in pain and hyperalgesia," *Current Topics in Medicinal Chemistry*, vol. 5, no. 6, pp. 547–555, 2005.

[34] J. F. MacDonald, M. F. Jackson, and M. A. Beazely, "Hippocampal long-term synaptic plasticity and signal amplification of NMDA receptors," *Critical Reviews in Neurobiology*, vol. 18, no. 1-2, pp. 71–84, 2006.

[35] H. Möhler, U. Rudolph, D. Boison, P. Singer, J. Feldon, and B. K. Yee, "Regulation of cognition and symptoms of psychosis: focus on GABA(A) receptors and glycine transporter 1," *Pharmacology Biochemistry and Behavior*, vol. 90, no. 1, pp. 58–64, 2008.

[36] J. S. Serrano, M. I. Serrano, M. R. Guerrero, R. Ruiz, and J. Polo, "Antinociceptive effect of taurine and its inhibition by naxolone," *General Pharmacology*, vol. 21, no. 3, pp. 333–336, 1990.

[37] C. S. Hornfeldt, D. H. Smullin, C. D. Schamber, X. Sun, and A. A. Larson, "Antinociceptive effects of intrathecal taurine and calcium in the mouse," *Life Sciences*, vol. 50, no. 24, pp. 1925–1934, 1992.

[38] I. Belfer, E. Davidson, A. Ratner, E. Beery, Y. Shir, and Z. Seltzer, "Dietary supplementation with the inhibitory amino acid taurine suppresses autotomy in HA rats," *NeuroReport*, vol. 9, no. 13, pp. 3103–3107, 1998.

[39] I. S. Lee, W. M. Renno, and A. J. Beitz, "A quantitative light and electron microscopic analysis of taurine-like immunoreactivity in the dorsal horn of the rat spinal cord," *Journal of Comparative Neurology*, vol. 321, no. 1, pp. 65–82, 1992.

[40] D. A. Bereiter, S. Shen, and A. P. Benetti, "Sex differences in amino acid release from rostral trigeminal subnucleus caudalis after acute injury to the TMJ region," *Pain*, vol. 98, no. 1-2, pp. 89–99, 2002.

[41] S. A. Park, H. Yin, J. P. Bhattarai et al., "Postnatal change of GluR5 kainate receptor expression in the substantia gelatinosa neuron of the trigeminal subnucleus caudalis in mice," *Brain Research*, vol. 1346, pp. 52–61, 2010.

[42] N. Tokutomi, M. Kaneda, and N. Akaike, "What confers specificity on glycine for its receptor site?" *British Journal of Pharmacology*, vol. 97, no. 2, pp. 353–360, 1989.

[43] N. Hussy, C. Deleuze, A. Pantaloni, M. G. Desarménien, and F. Moos, "Agonist action of taurine on glycine receptors in rat supraoptic magnocellular neurones: possible role in osmoregulation," *Journal of Physiology*, vol. 502, no. 3, pp. 609–621, 1997.

[44] Z. Tang, Y. Lu, and L. Chen, "Developmental stability of taurine's activation on glycine receptors in cultured neurons of rat auditory cortex," *Neuroscience Letters*, vol. 430, no. 1, pp. 54–59, 2008.

[45] N. Y. Song, H. B. Shi, C. Y. Li, and S. K. Yin, "Interaction between taurine and GABA(A)/glycine receptors in neurons of the rat anteroventral cochlear nucleus," *Brain Research*, vol. 1472, pp. 1–10, 2012.

[46] M. Puopolo, I. Kratskin, and O. Belluzzi, "Direct inhibitory effect of taurine on relay neurones of the rat olfactory bulb in vitro," *NeuroReport*, vol. 9, no. 10, pp. 2319–2323, 1998.

[47] O. Belluzzi, M. Puopolo, M. Benedusi, and I. Kratskin, "Selective neuroinhibitory effects of taurine in slices of rat main olfactory bulb," *Neuroscience*, vol. 124, no. 4, pp. 929–944, 2004.

[48] Z.-Y. Wu and T. Xu, "Taurine-evoked chloride current and its potentiation by intracellular Ca^{2+} in immature rat hippocampal CA1 neurons," *Amino Acids*, vol. 24, no. 1-2, pp. 155–161, 2003.

[49] B. M. Stell and I. Mody, "Receptors with different affinities mediate phasic and tonic GABA(A) conductances in hippocampal neurons," *The Journal of Neuroscience*, vol. 22, no. 10, p. RC223, 2002.

[50] D. W. Cope, S. W. Hughes, and V. Crunelli, "GABA(A) receptor-mediated tonic inhibition in thalamic neurons," *The Journal of Neuroscience*, vol. 25, no. 50, pp. 11553–11563, 2005.

[51] T. Shirasaki, M. R. Klee, T. Nakaye, and N. Akaike, "Differential blockade of bicuculline and strychnine on GABA- and glycine-induced responses in dissociated rat hippocampal pyramidal cells," *Brain Research*, vol. 561, no. 1, pp. 77–83, 1991.

[52] T. Horikoshi, A. Asanuma, K. Yanagisawa, K. Anzai, and S. Goto, "Taurine and beta-alanine act on both GABA and glycine receptors in Xenopus oocyte injected with mouse brain messenger RNA," *Brain Research*, vol. 464, no. 2, pp. 97–105, 1988.

[53] Z. Jiang, K. Krnjević, F. Wang, and J. H. Ye, "Taurine activates strychnine-sensitive glycine receptors in neurons freshly isolated from nucleus accumbens of young rats," *Journal of Neurophysiology*, vol. 91, no. 1, pp. 248–257, 2004.

[54] P. Pfeiffer, D. Graham, and H. Betz, "Purification by affinity chromatography of the glycine receptor of rat spinal cord," *The Journal of Biological Chemistry*, vol. 257, no. 16, pp. 9389–9393, 1982.

[55] P. Legendre, "The glycinergic inhibitory synapse," *Cellular and Molecular Life Sciences*, vol. 58, no. 5-6, pp. 760–793, 2001.

[56] J. Grudzinska, R. Schemm, S. Haeger et al., "The β subunit determines the ligand binding properties of synaptic glycine receptors," *Neuron*, vol. 45, no. 5, pp. 727–739, 2005.

[57] M. H. Cheng, M. Cascio, and R. D. Coalson, "Homology modeling and molecular dynamics simulations of the α1 glycine receptor reveals different states of the channel," *Proteins*, vol. 68, no. 2, pp. 581–593, 2007.

[58] I. Pribilla, T. Takagi, D. Langosch, J. Bormann, and H. Betz, "The atypical M2 segment of the β subunit confers picrotoxinin resistance to inhibitory glycine receptor channels," *The EMBO Journal*, vol. 11, no. 12, pp. 4305–4311, 1992.

[59] P. Jiang, Y. Kong, X. Zhang, W. Wang, C. Liu, and T. Xu, "Glycine receptor in rat hippocampal and spinal cord neurons as a molecular target for rapid actions of 17-β-estradiol," *Molecular Pain*, vol. 5, article 2, 2009.

[60] T. Takazawa and A. B. MacDermott, "Glycinergic and GABAergic tonic inhibition fine tune inhibitory control in regionally distinct subpopulations of dorsal horn neurons," *Journal of Physiology*, vol. 588, no. 14, pp. 2571–2587, 2010.

[61] P. J. Whiting, "GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery?" *Drug Discovery Today*, vol. 8, no. 10, pp. 445–450, 2003.

[62] E. R. Korpi, R. M. Mihalek, S. T. Sinkkonen et al., "Altered receptor subtypes in the forebrain of GABA(A) receptor δ subunit-deficient mice: recruitment of γ2 subunits," *Neuroscience*, vol. 109, no. 4, pp. 733–743, 2002.

[63] W. Sieghart and G. Sperk, "Subunit composition, distribution and function of GABA(A) receptor subtypes," *Current Topics in Medicinal Chemistry*, vol. 2, no. 8, pp. 795–816, 2002.

[64] P. Somogyi, J.-M. Fritschy, D. Benke, J. D. B. Roberts, and W. Sieghart, "The γ2 subunit of the GABA(A) receptor is concentrated in synaptic junctions containing the α1 and β2/3 subunits in hippocampus, cerebellum and globus pallidus," *Neuropharmacology*, vol. 35, no. 9-10, pp. 1425–1444, 1996.

[65] W. Wei, N. Zhang, Z. Peng, C. R. Houser, and I. Mody, "Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus," *The Journal of Neuroscience*, vol. 23, no. 33, pp. 10650–10661, 2003.

[66] A. Semyanov, M. C. Walker, D. M. Kullmann, and R. A. Silver, "Tonically active GABA(A) receptors: modulating gain and maintaining the tone," *Trends in Neurosciences*, vol. 27, no. 5, pp. 262–269, 2004.

[67] M. Farrant and Z. Nusser, "Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors," *Nature Reviews Neuroscience*, vol. 6, no. 3, pp. 215–229, 2005.

[68] J. P. Bhattarai, S. A. Park, J. B. Park et al., "Tonic extrasynaptic GABA(A) receptor currents control gonadotropin-releasing hormone neuron excitability in the mouse," *Endocrinology*, vol. 152, no. 4, pp. 1551–1561, 2011.

[69] C. J. A. Game and D. Lodge, "The pharmacology of the inhibition of dorsal horn neurones by impulses in myelinated cutaneous afferents in the cat," *Experimental Brain Research*, vol. 23, no. 1, pp. 75–84, 1975.

[70] M. Yoshimura and S. Nishi, "Blind patch-clamp recordings from substantia gelatinosa neurons in adult rat spinal cord slices: pharmacological properties of synaptic currents," *Neuroscience*, vol. 53, no. 2, pp. 519–526, 1993.

[71] H. Ripps and W. Shen, "Review: taurine: a "very essential" amino acid," *Molecular Vision*, vol. 18, pp. 2673–2686, 2012.

[72] K. Matsumoto, E. H. Lo, A. R. Pierce, E. F. Halpern, and R. Newcomb, "Secondary elevation of extracellular neurotransmitter amino acids in the reperfusion phase following focal cerebral ischemia," *Journal of Cerebral Blood Flow and Metabolism*, vol. 16, no. 1, pp. 114–124, 1996.

[73] Y. Uchiyama-Tsuyuki, H. Araki, T. Yae, and S. Otomo, "Changes in the extracellular concentrations of amino acids in the rat striatum during transient focal cerebral ischemia," *Journal of Neurochemistry*, vol. 62, no. 3, pp. 1074–1078, 1994.

[74] M. A. M. Silva, G. M. A. Cunha, G. S. B. Viana, and V. S. N. Rao, "Taurine modulates chemical nociception in mice," *Brazilian Journal of Medical and Biological Research*, vol. 26, no. 12, pp. 1319–1324, 1993.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2013, Article ID 654257, 19 pages
http://dx.doi.org/10.1155/2013/654257

Review Article

Ionotropic Glutamate Receptors and Voltage-Gated Ca^{2+} Channels in Long-Term Potentiation of Spinal Dorsal Horn Synapses and Pain Hypersensitivity

Dong-ho Youn,[1] Gábor Gerber,[2] and William A. Sather[3]

[1] *Department of Oral Physiology, School of Dentistry, Kyungpook National University, 188-1 Samduck-2, Chung-gu, Daegu 700-412, Republic of Korea*
[2] *Department of Anatomy, Histology and Embryology, Semmelweis University, Tüzoltó Utca 58, Budapest, Hungary*
[3] *Department of Pharmacology, University of Colorado School of Medicine, Mail Stop 8315, 12800 E. 19th Avenue, P18-7104, Aurora, CO 80045, USA*

Correspondence should be addressed to Dong-ho Youn; dyoun@knu.ac.kr

Received 11 July 2013; Revised 27 August 2013; Accepted 27 August 2013

Academic Editor: Clive Bramham

Over the last twenty years of research on cellular mechanisms of pain hypersensitivity, long-term potentiation (LTP) of synaptic transmission in the spinal cord dorsal horn (DH) has emerged as an important contributor to pain pathology. Mechanisms that underlie LTP of spinal DH neurons include changes in the numbers, activity, and properties of ionotropic glutamate receptors (AMPA and NMDA receptors) and of voltage-gated Ca^{2+} channels. Here, we review the roles and mechanisms of these channels in the induction and expression of spinal DH LTP, and we present this within the framework of the anatomical organization and synaptic circuitry of the spinal DH. Moreover, we compare synaptic plasticity in the spinal DH with classical LTP described for hippocampal synapses.

1. Introduction

Long-term potentiation (LTP), an increase in the strength of synaptic transmission between neurons, has been proposed as a cellular model of learning and memory formation. Since LTP was first described for the dentate area of the hippocampal formation [1], data pertinent to mechanisms of LTP have been abundantly accumulated in diverse synapses of hippocampus and other brain areas. In contrast, investigation of LTP in the spinal dorsal horn (DH) [2] is more recent, beginning twenty years after the first description of LTP in the hippocampus, and spinal DH LTP has focused largely upon the synapses formed by primary sensory afferent fibers, because these synapses are the first checkpoint for pain signals entering the central nervous system (CNS). At these primary afferent synapses, LTP has been thought to be a cellular correlate of pain hypersensitivity and as such has been proposed as a potential target for therapeutic treatments of chronic pain.

Neurons in the spinal DH, consisting of superficial (laminae I and II) and deep (laminae III–VI) DH, receive synaptic inputs from primary afferent fibers, their cell bodies located within dorsal root ganglion (DRG) as well as those from other DH neurons, or neurons in other higher brain areas. The spinal DH neurons are considered as secondary neurons because peripheral somatosensory signals conveyed by primary sensory DRG neurons first reach these neurons. Synapses formed in these DH neurons mostly use glutamate for excitatory transmission. Generally, ionotropic glutamate receptors selectively activated by the artificial agonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) support the largest component of glutamatergic excitatory synaptic transmission in the CNS, while the *N*-methyl-D-aspartate (NMDA) receptor subtype is most important in the induction of synaptic plasticity, including LTP (*see below*). In addition to ligand-gated excitatory ion channels, DH neurons express various types of voltage-gated ion channels that generally contribute to neuronal excitability. Among

the voltage-gated ion channels, voltage-gated Ca^{2+} channels (VGCCs) have been found to be involved in the control of synaptic plasticity, owing to their control of Ca^{2+} influx into both presynaptic nerve terminals and postsynaptic domains of neurons.

In this paper, we review the contributions of these two classes of ion channels to LTP in the spinal DH area. To provide a context for interpretation of the role of these channels in LTP, we first briefly discuss the anatomical organization and synaptic circuitry of the spinal DH and also consider synaptic transmission and plasticity in the spinal DH. For the sake of brevity, this review does not consider the roles of other types of ion channels in plasticity and pain, nor does it focus upon downstream signaling pathways known to be critical for LTP.

2. The Spinal Cord Dorsal Horn

2.1. Anatomical Organization. The DH of the spinal cord can be subdivided into six distinct layers (laminae I–VI) in the dorsal-ventral direction of the gray matter, which was first proposed in cat [3] as well as in rat [4]. The Rexed laminae I and II consist of superficial spinal DH [5], and laminae III–VI are frequently called deeper layer of the spinal cord. Due to concentrated small neurons and their processes plus a relative small number of myelinated axons, the lamina II is observed as a translucent band under the naked eye or light microscope and is called "substantia gelatinosa (SG)" [4, 6]. Lamina VI exists only in the cervical and lumbosacral enlargements [3]. Generally, the spinal DH consists of the central terminals of primary sensory neurons, projection neurons, intrinsic DH neurons, and descending nerve fibers from the brainstem and other higher brain structures. The cell bodies of the primary sensory neurons are located in the DRG. Each ganglion cell sends an axon that branches into a peripheral process and a central process. The peripheral process contributes to a peripheral nerve and terminates peripherally as a sensory receptor. The central process enters into the spinal cord through a dorsal root and further branches to numerous collaterals. Together, these two processes form primary afferent fibers that transmit encoded information from periphery to the spinal cord or trigeminal nuclei of the brain stem.

Although primary afferent fibers give off most of their collaterals to the segment of the spinal cord that they enter, they also spread in the rostrocaudal direction. The distribution of primary afferent fibers in the spinal DH is in an orderly way based on fiber size, which affects conduction velocity and sensory modality [7]. Most fine myelinated (Aδ; conduction velocity, 1–1.5 to 5–10 m/sec) or unmyelinated (C; <1–1.5 m/sec) primary afferent fibers end predominantly in laminae I and II, although a few reach laminae III–VI [8, 9]. In detail, high threshold Aδ mechanoreceptors terminate in laminae I and V, while low threshold Aδ mechanoreceptors only terminate in lamina III [9]. Most large cutaneous afferents (Aβ; >5–10 m/sec), which function as low threshold mechanoreceptors, have a characteristic pattern of termination in the deeper laminae (III–VI) of the DH [10]. Cutaneous C fibers, occupying ~80% of cutaneous primary afferent fibers [11] and the majority of which being high-threshold polymodal nociceptors in the rat [12], terminate in lamina II [13–15], although there is also a contribution to lamina I [16]. Based on neurochemical markers, the high-threshold C fibers can be divided into two major groups: peptidergic and nonpeptidergic [7]. Peptidergic C fibers are nociceptors [17] and contain neuropeptides such as calcitonin gene-related peptide (CGRP) and/or substance P and express TrkA or transient receptor potential (TRP) V1 [18]. The peptidergic substance P-containing C fibers ends mainly in lamina I and the outer layer of lamina II (IIo). It is estimated in lumbar DRG of rat that approximately half of the C fibers are peptidergic [19]. Other high-threshold C fibers do not contain peptides, but most of them can be revealed by their ability to bind the lectin Bandeiraea simplicifolia isolectin B4 (IB4) [20], and a subpopulation of the nonpeptidergic C fibers can be defined by Mas-related G-protein-coupled receptor member D (MrgprD), a sensory neuron-specific G-protein-coupled receptor [21]. Although the function of nonpeptidergic C fibers is poorly understood, this population also includes many nociceptors [22, 23] and is different from the peptidergic C fiber because ablation of the MrgprD afferents in adult mice results in a selective loss of sensitivity to noxious mechanical (but not thermal) stimuli [7, 24].

Beside the high-threshold C fibers, it should be pointed out that there are low-threshold mechanosensitive C fibers, which are nonpeptidergic and innervate specific types of hair follicles [25]. Interestingly, this type of nonpeptidergic C fiber expresses neither IB4 nor MrgprD but exclusively expresses tyrosine hydroxylase, the enzyme catalyzing L-3,4-dihydroxyphenylalanine production and participates in forming narrow unique columns in the spinal DH with other low-threshold Aδ and Aβ fibers [25].

2.2. Synaptic Circuitry. The gate control theory, proposed by Melzack and Wall [26], illustrates how pain signals are transmitted to higher brain areas via the spinal DH. In this theory, inhibitory SG neurons control presynaptically both large- and small-diameter fibers, presumably corresponding to, respectively, A and C fibers, and these in turn innervate the transmission system. Therefore, activation of SG neurons by large-diameter fibers attenuates signals conveyed via both fiber types to transmission system neurons, which corresponds to gate closing; in contrast, inhibition of SG neurons by small diameter fibers opens the gate for transmission of pain information. Although the theory proposes a prominent role for SG neurons in gating pain transmission, it assumes only a single type of SG neurons, which is certainly incorrect in regard to the synaptic organization of the DH [27]; rather, recent data demonstrate that different subtypes of SG neurons are present in spinal DH and that these subtypes make distinct contributions to the function of the complex synaptic network of the spinal DH. Thus, based on recent morphological and electrophysiological studies in the spinal DH, we attempt to assign various SG neurons to three functionally different SG neurons: inhibitory SG (iSG) neurons, excitatory SG (eSG) neurons, and transmission system-inhibiting (tiSG) SG neurons (Figure 1). Large-diameter A fibers directly, or

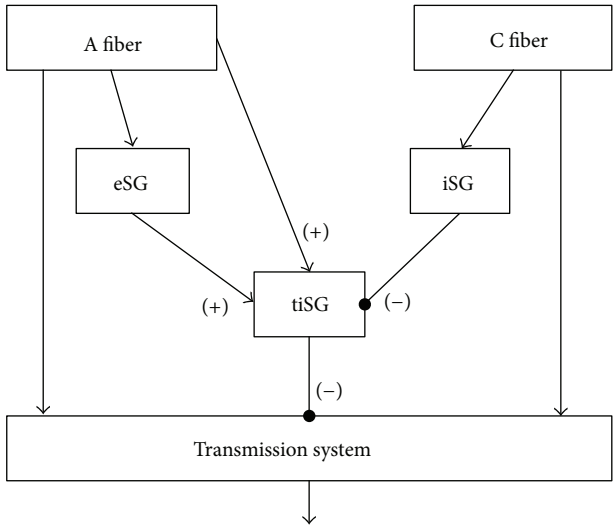


Figure 1: A diagram modified from the gate control theory. Both primary afferent A and C fibers directly target the transmission system that conveys the pain signals from the spinal dorsal horn to the higher brain areas. However, both fibers differentially innervate to the substantia gelatinosa (SG) neurons in the spinal DH. Although polysynaptic inputs are possible in all SG neurons from primary afferent fibers and other SG neurons, monosynaptic inputs from A fibers reach the excitatory SG neurons (eSG) and the transmission-inhibiting SG neurons (tiSG), while those from C fibers only go into the inhibitory SG neurons (iSG), not the itSG directly. The tiSG neurons receive the excitatory synaptic inputs from the eSG and the inhibitory synaptic inputs from the iSG. The main function of tiSG is inhibiting the transmission system, both presynaptically (in the gate control theory) and postsynaptically (in this diagram). In this way, the activation and inhibition of SG neurons (here, called tiSG) by large-diameter and small-diameter fibers, respectively, are possible, shown in the gate control theory.

indirectly through eSGs, activate tiSG neurons, whereas small-diameter C fibers necessarily activate iSG neurons to inhibit the tiSG neurons. The transmission system is composed of projection neurons that send pain information to higher brain centers. In future works, it will be important to more completely describe the synaptic organization of the DH, and it will be important to carefully define the three basic classes of SG neurons.

The primary candidates for iSG neurons are γ-aminobutyric acid- (GABA-) ergic neurons in the spinal DH, an electrophysiologically heterogeneous group [28, 29] that make up approximately 30% of neurons in lamina II (SG) of the spinal cord [30]. It has been suggested, using combined single/paired whole-cell patch-clamp recordings and biocytin labeling, that ~75% of all SG neurons fall into five groups that differ in firing behavior and other electrophysiological properties and most prominently, in the structure of their dendritic arbors: islet, radial, central, medial-lateral, and vertical cells [31–36]. Among these groups, islet cells are exclusively GABAergic [37, 38] and receive monosynaptic input from C fibers and polysynaptic input from Aδ fibers [33, 35, 36]. The GABAergic nature of islet cells corresponds with the finding that, among SG neurons expressing GFP (driven by the upstream regulatory sequence of the gene encoding the GABA synthetic enzyme, glutamic acid decarboxylase (GAD) 67), 62% have islet-type morphology [29]. Because the islet cells do not directly target projection neurons [34] yet do influence the pain transmission system through other SG neuronal types, islet cells are apparently one of the major sources of SG neurons that act as iSG neurons (Figure 1).

In addition to the islet-type of SG neuron, tonically firing neurons with central-type dendritic arbors are also likely GABAergic [32]. Central neurons receiving GABAergic input from islet cells and glutamatergic input from C fibers are divided into either tonically firing neurons or transiently firing neurons [31] and are both excitatory and inhibitory [32–35]. The tonically firing central neurons synapse on vertical neurons and recurrently, on islet cells, identifying this subtype of central neuron as an additional type of iSG neuron.

As noted above, 30% of SG neurons are GABAergic so that ~2/3 of SG neurons are glutamatergic; eSG neurons are drawn from this large pool of excitatory interneurons. The eSG neurons likely include vertical and radial SG neurons because they predominantly receive monosynaptic inputs from A fibers [34, 36, 38]. In contrast, it is known that central-type SG neurons do not receive monosynaptic inputs from A fibers, thus ruling out central neurons as candidate eSG neurons.

On the other hand, considerably less is known about the types of GABAergic SG neurons that make up the tiSG circuit component, which synapse on projection neurons in lamina I and deeper laminae [7]. Neurokinin (NK) 1-positive projection neurons in laminae III-IV are known to receive inputs from GABAergic neurons that contain neuropeptide Y (NPY) [39]. Although classification of SG neuronal types by arborization pattern and electrophysiological properties remains incomplete, because NPY-expressing neurons comprise half of the GABAergic neurons of laminae I and II [40], we suggest that these NPY-positive neurons inhibit the transmission system and thus may act as tiSG neurons (Figure 1). Therefore, careful identification of the types of (1) primary afferent fibers and (2) local interneurons that send inputs to NPY-positive, GABAergic neurons, would greatly clarify the synaptic circuitry that contributes to gating of pain signals. Evidence for another type of tiSG neuron has also been obtained: "giant marginal" projection neurons, which lack NK1 receptors and express the glycine receptor-associated protein gephyrin [41], are richly contacted by GABAergic boutons that contain nitric oxide synthase (NOS) but not NPY [41]. Here again, the neuronal morphology needs to be defined for this NOS-positive neuron. Altogether, clear understanding of the synaptic circuitry underlying gating of pain signals will require a more complete description of the pattern of SG neuron connectivity with GABAergic, NPY- or NOS-positive neurons.

2.3. Synaptic Transmission. At chemical synapses in the CNS, neurotransmitters released from presynaptic nerve terminals generate graded analogue signals through the opening of ligand-gated ion channels on the plasma membrane of postsynaptic neurons. Whereas each presynaptic neuron

possesses the biochemical machinery to release only one type of neurotransmitter, which can be either excitatory or inhibitory, individual postsynaptic neurons express a variety of ligand-gated ion channels that respond to different neurotransmitters so that postsynaptic neurons can, for example, exhibit both excitatory and inhibitory synaptic potentials. In the CNS, glutamate generates fast excitatory synaptic signals in postsynaptic neurons by opening any of three types of ligand-gated glutamate receptor ion channels: based on their pharmacology and structural homology, these are known as AMPA receptors (subunit: GluA1-GluA4), kainate (subunit: GluK1-GluK5) receptors, and NMDA receptors (subunit: GluN1, GluN2A-GluN2D, GluN3A, and GluRN3B) [42].

It is known from early studies of the spinal DH that glutamate-mediated fast excitatory synaptic transmission involves activation of postsynaptic ionotropic glutamate receptors [43–46]. AMPA receptors mediate the large early component of fast excitatory synaptic postsynaptic responses, whereas the more slowly opened NMDA receptors contribute only to the later component of excitatory postsynaptic responses [2, 45, 46]. Fast synaptic transmission mediated by kainate receptors is relatively small and produces slowly decaying synaptic currents [47, 48]. Although most spinal DH synapses use all three classes of ionotropic glutamate receptors, some synapses possess only NMDA receptors; these are known as "silent synapses" because, lacking AMPA-receptor-driven postsynaptic depolarization, the glutamate-activated NMDA receptors at these synapses remain blocked by Mg^{2+} and thus fail to generate a synaptic signal [49, 50].

Although inhibitory transmission falls outside the scope of the present review, we note that many interneurons in the spinal DH release GABA and glycine, which provide fast inhibitory transmission that is an essential feature of spinal DH circuits. Most GABAergic neurons also release glycine in the spinal DH [30, 51, 52], but GABA-mediated transmission can be distinguished from glycine-mediated transmission based on the decay kinetics of synaptic responses [53, 54].

2.4. Synaptic Plasticity. Strength of synaptic transmission in the CNS is not constant; rather, it is subject to up- or downmodulation as a consequence of patterns of presynaptic and/or postsynaptic activity. Such activity-dependent changes in synaptic strength are accomplished, in part, through long-term modulation of the properties and numbers of ion channels that mediate, affect, or respond to synaptic activity. LTP—a persistent increase in the strength of synaptic transmission [1]—can be induced by tetanic stimulation, pairing of presynaptic activity with postsynaptic depolarization, coincidence between presynaptic release of glutamate and postsynaptic depolarization, and pharmacological treatments that increase excitatory postsynaptic responsivity. Since the initial discovery of LTP, molecular and cellular mechanisms subserving this kind of plasticity have been worked out most clearly for the canonical form of NMDA receptor-dependent LTP that is found at Schaffer collateral-commissural synapses onto pyramidal neurons in the CA1 region of hippocampus. Important elements that have been identified include channel phosphorylation by protein kinases such as protein kinase A (PKA), protein kinase C (PKC), and Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) [55–59]; consequent increases in channel opening probability and single-channel conductance [60, 61]; subunit-specific trafficking of postsynaptic AMPA receptors [62–64] to the subsynaptic membrane; and changes in glutamate release, both in probability [65] and quantal content [66] at presynaptic terminals [67]. The cellular processes most carefully worked out for hippocampal LTP [68] are generally thought to provide a fundamental basis for information processing and storage throughout CNS and particularly for learning and memory in the hippocampus [69–72].

In the spinal cord DH, early studies revealed that repetitive stimulation of dorsal root or peripheral nerve produces LTP at primary afferent synapses [2, 73, 74]. In addition to involvement of NMDA receptors and postsynaptic Ca^{2+} that is typical of LTP induction in hippocampus [2, 75], spinal DH studies have identified roles for NK1 [75, 76], group I metabotropic glutamate [77], and opioid [78] receptors in the induction and expression of LTP [2].

Some patterns of synaptic activity can cause a decrease in synaptic strength, referred to as "long-term depression (LTD)" [79]. This type of synaptic plasticity has also been extensively studied in various CNS regions, most particularly in the context of certain forms of information processing in the hippocampus [80, 81] and also of motor learning in cerebellum [82]. Although both high-frequency stimulation (HFS) and low-frequency stimulation (LFS) can induce LTD in the spinal DH, protein phosphatases play a role only in the induction of HFS-induced LTD [83] but not in that of LFS-LTD [84] in this area.

Because spinal LTP and LTD may play critical roles in hyperalgesia and allodynia [85, 86] and the activation of high-threshold C fibers is important to mediate many type of hyperalgesia, C fiber-mediated field potentials have been the subject of many LTP studies. LTP of C fiber-evoked field potentials is reliably produced by HFS of peripheral nerves (>3 hours), and is dependent upon the activation of NMDA receptors [75]; interestingly, LFS at C fiber intensity also induces LTP under certain conditions [87, 88]. Moreover, C fiber-mediated LTP can be induced by noxious stimulation or injury [89], revealing a contribution of this form of synaptic plasticity to induction of hyperalgesia. Although the loci of mechanisms underlying the LTP of C fiber-evoked field potential are difficult to clarify, the induction and/or maintenance of this type of LTP involve many channels and signaling molecules, including NMDA and NK1 receptors [75], N-type and P/Q-type VGCCs [90], TRPV1 channels [91], the EphB receptor tyrosine kinase [92], ryanodine receptors [93, 94], nitric oxide [95, 96], and many inflammatory agents [97].

Spinal LTP has also been studied using single (whole) cell recordings of primary afferent stimulation-evoked excitatory postsynaptic potentials or currents (EPSPs and EPSCs, resp.). In this case, Aδ fiber- and C fiber-mediated synaptic responses can be distinguished according to stimulus intensity and conduction velocity, which is advantageous in elucidating primary afferent-dependent mechanisms [87, 98, 99]

or the locus of induction of LTP [93, 100]. In addition, whole cell recordings are advantageous for study of LTP in particular types of DH neurons. Combining whole cell recording from DH neurons with injection of retrograde tracers (e.g., DiI) into the parabrachial (PB) area or periaqueductal gray (PAG) has allowed researchers to determine that HFS induces LTP of C fiber-mediated EPSCs in lamina I neurons that project to PB area but not in those that project to PAG [87]; in contrast, LFS induces LTP in lamina I neurons that project to PAG area. LTP of C fiber-EPSCs by HFS in the PB-projecting lamina I neurons requires NK1 receptor-mediated signaling and activation of T-type VGCCs [99]. LFS-induced LTP of C fiber EPSCs in the PAG-projecting lamina I neurons requires nitric oxide signals [87, 93], the latter generated in response to intracellular Ca^{2+} rises that are slower in onset and prolonged in comparison to the kinetics of Ca^{2+} rises required to elicit LTP in PB-projecting lamina I neurons [87].

In CA1 of hippocampus, LTP of EPSCs can be alternatively induced by the coordinated activity of presynaptic fibers and postsynaptic neurons, which is characteristic of spike-timing [101] or pairing protocols [102] in the hippocampus. The spike-timing protocol requires low-frequency postsynaptic spikes timed within 10 ms of the onset of synaptic responses, while the pairing protocol involves persistent postsynaptic depolarization (0~+30 mV) during repetitive low-frequency presynaptic stimulation. These protocols have been applied to study LTP of EPSCs in the spinal DH as well, in an effort to overcome the low rate of success (~50%) observed for the induction of LTP by HFS [2]. Spike-timing dependent LTP is interestingly dependent on expression of the GluK2 (previously known as GluR6) kainate receptor subunit [48], along with activation of NMDA receptors and elevation of intracellular Ca^{2+} [103]. In spinal DH, LTP of EPSCs by the pairing protocol requires activation of extracellular signal-regulated kinase (ERK) [104].

Considering the mixture of excitatory and inhibitory interneurons and the complex synaptic circuitry in lamina II of the spinal DH (see above), the contribution of LTP to hyperalgesia will necessarily depend upon which synapses in the DH circuitry specifically undergo LTP. It is therefore critical that LTP and its pathophysiological role in pain hypersensitivity be pursued at identified types of SG neuron synapses. For example, hyperalgesia may be produced by LTP of excitatory interneurons that synapse upon neurons that, in turn, project to brain areas involved in nociception. Alternatively, hyperalgesia may be reduced by LTP of synapses onto inhibitory interneurons that target projection neurons. Hence, careful identification of the specific subtype of neuron studied will be essential to better understand the roles of spinal DH LTP in hyperalgesia and allodynia [85].

3. Contribution of Ionotropic Glutamate Receptors to LTP in the Spinal DH

3.1. AMPA Receptors. AMPA receptors consist of homo- and heterotetrameric assemblies of GluA1, 2, 3, and 4 subunits, with different assemblies of AMPA receptor subunits exhibiting distinct functional behaviors [42, 105]. Among the GluA subunits, transcripts encoding GluA2 are subject to RNA editing at position 586, which results in replacement of the neutral glutamine (Q) residue found in all other GluA subunits with a positively charged arginine (R). Position 586 is located in transmembrane segment 2 (M2), which forms the lining of the ion permeation pathway through the receptor; an arginine at this position decreases the receptor's Ca^{2+} permeability and also confers linear current-voltage behavior. AMPA receptors lacking GluA2 subunits are significantly more Ca^{2+}-permeable, exhibiting a Ca^{2+} permeability ratio (P_{Ca}/P_{Na}) of 3, and they also display strong inward rectification in their current-voltage relationships [105, 106].

Because AMPA receptors are the main mediators of excitatory synaptic transmission in the CNS, they are generally considered as the final target for induction and expression of LTP, rather than as inducers or regulators. Thus, principal endpoints in LTP are phosphorylation and trafficking of specific AMPA receptor subunit subtypes, along with changes in AMPA receptor conductance [42, 105]. The GluA1 subunit, for example, can undergo phosphorylation of Ser831 by CaMKII [107] and PKC [108] and of Ser845 by PKA [108], which contributes to induction of LTP by changing the open probability and single-channel conductance of AMPA receptors containing this subunit [42]. In regard to membrane trafficking of AMPA receptors, the induction mechanism for LTP in hippocampal CA1 pyramidal neurons [64] includes increased incorporation of GluA1/GluA2-containing AMPA receptors into the synaptic surface membrane [62, 109]; however, subsequent work suggests that the newly incorporated AMPA receptors are in fact homotetrameric GluA1 complexes [110]. In accordance with these studies, the induction of LTP at hippocampal CA1 synapses is impaired in mice deficient in the GluA1 subunit [111]. Surface membrane incorporation of homomeric GluA1 receptors may result in the replacement of preexisting GluA2-containing AMPA receptors, thereby increasing the net Ca^{2+}-permeability of the AMPA receptor population in the postsynaptic surface membrane [112]. Increased Ca^{2+} influx via GluA2-lacking, Ca^{2+}-permeable AMPA receptors, is directly related to enhancement of LTP [113, 114].

Trafficking of AMPA receptors requires their interaction with transmembrane AMPA receptor regulatory proteins (TARPs) [115, 116]. Interaction of TARPs with AMPA receptors prevents AMPA receptor degradation [117], and subsequent interaction of AMPA receptors with PSD-95 results in translocation of AMPA receptors from the perisynaptic region into synaptic sites [118]. In contrast to these studies, a recent study has found that the GluA1 C-terminal tail, critical for GluA1 trafficking [109, 110], is not required for LTP [119]. This has led to the suggestion that a reserve pool of AMPA receptors, regardless of their subunit composition, is relied upon for LTP. Further studies are needed to provide a more comprehensive picture of the mechanism and role of AMPA receptor trafficking in hippocampal LTP. In addition, studies of this process in spinal DH LTP remain to be carried out.

Ca^{2+}-permeable AMPA receptors are expressed in inhibitory interneurons [120] of lamina I and the outer layer of lamina II [121], the laminae which receive synaptic input

primarily from nociceptive C fiber afferents. Ca^{2+}-permeable AMPA receptors in layer I and II DH neurons are activated by synaptic input [122], raising the possibility that these channels play a special role in mediating sensory input by unmyelinated fibers [123]. Using GluA2 knockout mice, it has been shown that Ca^{2+}-permeable AMPA receptors enhance HFS-evoked LTP and mediate induction of NMDA receptor-independent LTP at primary afferent-DH neuron synapses [98], which suggests that Ca^{2+}-permeable AMPA receptors contribute significantly to LTP in the spinal DH and may substitute for NMDA receptors in LTP induction. In contrast to NMDA receptors, Ca^{2+}-permeable AMPA receptors allow Ca^{2+} influx at resting membrane potential, a potential merit for induction of synaptic plasticity.

3.2. NMDA Receptors. Functional NMDA receptors are heterotetrameric assemblies composed of two GluN1 subunits and either two GluN2 subunits or a combination of GluN2 and GluN3 subunits [42]. The glutamate binding sites are located in the GluN2 subunits [124] and the glycine binding sites in the GluN1 and GluN3 [124–126]. NMDA receptors are characterized by their high permeability to Ca^{2+} [127], voltage-dependent block by Mg^{2+} [128], and slow "activation/deactivation" kinetics [129]. NMDA receptor alternative splice variants exhibit subtle differences in functional properties, thereby fine-tuning the behavior of NMDA receptors in which they are incorporated [130]. For example, NMDA receptors containing GluN2A or GluN2B subunits display high-conductance channel openings and a high sensitivity to block by extracellular Mg^{2+}, whereas receptors composed of GluN2C or GluN2D subunits show low-conductance openings and lower sensitivity to Mg^{2+}. Moreover, GluN1/GluN2A-containing NMDA receptor currents deactivate rapidly (time constant of tens of milliseconds), whereas GluN1/GluN2D-containing NMDA receptor currents deactivate very slowly (time constant of several seconds) [131–133]. In addition, GluN3 can also coassemble with GluN1 [134–136] to form uniquely excitatory glycine receptors [136]. These distinctive properties may provide particular NMDA receptor subtypes with specific roles in excitatory synaptic transmission/plasticity and pathology.

The role of NMDA receptors in the induction of LTP is well established for various brain synapses, particularly the Schaffer collateral input to CA1 pyramidal neurons in hippocampus [68–70]. The activation requirements for NMDA receptors—agonist (glutamate) binding and postsynaptic depolarization—are well-matched to the "Hebbian" properties of LTP induction, namely, specificity, associativity, and cooperativity [69]. Further, several recent studies show that a proper subunit composition is essential for the induction [137–139] and expression [140, 141] of LTP. These results reflect the fact that specific NMDA receptor subunits are differentially phosphorylated by various protein kinases, such as src [142, 143] and also differentially interact with other accessory and regulatory proteins [144]. In keeping with the notion of a proper NMDA subunit composition in LTP, the association of active CaMKII with GluN2B is likely required for the induction of canonical LTP at Schaffer collateral synapses on CA1 neurons [145]. Downstream of these regulatory processes, NMDA receptor-dependent protein synthesis [146–148] is needed for the expression of LTP that persists beyond ~4 hours, referred to as late-LTP [149].

In the spinal DH, *in situ* hybridization or immunostaining has revealed high expression of GluN1 and GluN2D [150, 151] and lower levels of GluN2A and GluN2B [152, 153]. Electrophysiological measurements of conductance ratio have shown that lamina II GABAergic interneurons express both the GluN2A/GluN2B- and GluN2C/GluN2D-containing NMDA receptors, while excitatory lamina II interneurons express primarily GluN2A/GluN2B-containing receptors [154]. In addition, outside-out patch recordings of single channel currents have shown that, at least in extrasynaptic regions, both GluN1/GluN2B (high conductance; 57 pS) and GluN1/GluN2D (low conductance; 44 pS and 19 pS) are present on spinal DH neurons [155].

In the spinal DH, induction of nearly all forms of LTP is dependent on the NMDA receptors [86]. An early report showed that HFS (100 Hz) of primary afferent fibers at C fiber-activating intensity induces LTP of EPSPs in transverse spinal cord slices in vitro; LTP was absent in the presence of the NMDA receptor antagonist D-2-amino-5-phosphonovalerate (D-AP5) [2]. In addition, LTP induction at C fiber synapses also requires activation of NMDA receptors [73, 87, 99]. Recently, LFS (2 Hz at C-fiber intensity) of sciatic nerve has been shown to induce LTP of C fiber-evoked field potentials. This LFS-induced LTP is also prevented by an NMDA receptor antagonist, MK-801 in these experiments [156]. As expected, the noble anesthetic gas xenon, which has an inhibitory effect on NMDA receptors [157], prevents induction of LTP at C fiber synapses in intact rats [158]. LTP can also be induced by chemical means, for example, by perfusion of spinal cord slices with NMDA (+ postsynaptic depolarization) [159] or by perfusion of spinal cord segments with NMDA in spinalized, deeply anesthetized adult rat [75]. Taking together, these findings indicate that the NMDA receptor is required for induction of LTP in synapses of primary afferent fibers onto spinal DH neurons.

3.3. Kainate Receptors. Kainate receptors are tetramers assembled from combinations of five different types of subunits, termed GluK1-5 (formerly, GluR5-7 and KA1-2) [42, 105, 106, 160]. Each kainate receptor monomer possesses a ligand-binding site and a distinctive amino acid sequence that forms the channel lumen. Radioligand binding assays indicate that GluK1, 2, and 3 contribute to low-affinity kainate binding sites (K_D of 50–100 nM) [161], whereas GluK4 and 5 form high-affinity kainate binding sites (K_D of ~4–15 nM) [162, 163]. Structural variability of kainate receptors is conferred by alternative splicing and RNA editing [160]. Alternative splice variants have been found exclusively for GluK1 (GluK1-1, 1-2a, 1-2b, and 1-2c) [164] and GluK3 (GluR3a and 3b) subunits [165] in rat; however, the mouse GluK2 exists as two

splice variants that differ in their C-terminal domains [166]. RNA editing, as for GluA2 subunits, posttranscriptionally modifies a Q/R site in the M2 segment of GluK1 and GluK2 subunits. The Q-to-R substitution in GluK2 homomeric kainate receptors decreases Ca^{2+} permeability [167, 168] and increases Cl^{-} permeability [169], reduces unitary conductance, and transforms channels from inwardly rectifying to linear or slightly outwardly rectifying. Mice deficient in Q/R editing in GluK1 have been found to exhibit a reduction in kainate receptor-mediated currents in DRG neurons [170], although the responses of these animals to painful stimuli are unaffected. Besides the Q/R editing site, two additional positions prone to RNA editing have been identified in the GluK1 subunit: an isoleucine (I)/valine (V) site and a tyrosine (Y)/cysteine (C) site [171], both in the M1 segment. Although the additional editing sites may modulate Q/R site control of Ca^{2+} permeability, the mechanism of interaction among the three editing sites remains to be elucidated.

For NMDA receptor-independent LTP at the mossy fiber-CA3 synapse in hippocampus, there is disagreement regarding the role of kainate receptors [172]. A selective antagonist for GluK1-containing kainate receptors, LY382884, blocks the induction of mossy fiber LTP [173, 174], but conflictingly, mossy fiber LTP can be elicited in the presence of the AMPA/kainate receptor antagonist, CNQX [175, 176]. Knockout mice deficient in GluK2 subunits [177] display reduced mossy fiber LTP, but mice deficient in GluK1 possess normal LTP. Although much more work is needed, the results point to a potential role for kainate receptors in mossy fiber LTP, specifically in the NMDA receptor-independent form of LTP.

For synapses of primary afferents onto spinal DH neurons, fast EPSCs have been shown to be mediated by postsynaptic kainate receptors [47]. Kainate receptor-mediated EPSCs are much smaller in peak amplitude and slower in decay kinetics than those mediated by AMPA receptors. To date, the kainate receptor subunits mediating synaptic transmission have not been well-characterized. However, low expression of GluK1 subunits, moderate expression of GluK3 and GluK4, and strong expression of GluK5 have been found for spinal DH neurons; no expression of GluK2 has been detected [150, 178]. Despite the apparent absence of GluK2 subunits from DH neurons, kainate receptor-mediated whole cell current and synaptic potentials recorded from spinal DH neurons are significantly decreased in GluK2 mutant mice [48]. In addition to expression on postsynaptic membrane in spinal DH neurons, kainate receptors are also expressed on DRG neurons, including primary afferent presynaptic terminals within the DH [179, 180]. All kainate receptor subtypes are present in DRG neurons, with GluK1 expressed at an especially high level [178, 181–183]. GluK1- or GluK2-containing presynaptic kainate receptors modulates glutamatergic transmission at Aδ and C-fiber primary afferent-activated synapses in the spinal SG [184]. Interestingly, induction of LTP is impaired in GluK2 knockout mice, while the late phase of LTP is impaired in GluK1 mutant mice [48], indicating differential involvement of kainate receptor subunits in LTP of spinal DH neurons.

4. Voltage-Gated Ca^{2+} Channels That Contribute to LTP in Spinal DH and to Pain

Although receptors for L-glutamate, most commonly the NMDA receptor subtype, mediate induction and expression of LTP at many synapses in the brain, some forms of LTP at hippocampal CA1 synapses, such as late-phase LTP (L-LTP), require activation of L-type VGCCs. Other VGCCs are also involved in diverse ways in LTP, as discussed below.

VGCCs consist of a pore-forming transmembrane α_1 subunit and the auxiliary β subunit and $\alpha_2\delta$ subunit [185]. Based on sequence homology, the ten different α_1 subunits of VGCCs are grouped into three subfamilies: two high-voltage activated subfamilies, Ca_V 1-2, and one low-voltage activated family, Ca_V3 [186]. The Ca_V1 subfamily carries L-type Ca^{2+} current, and the family members are $Ca_V1.1$ (α_{1S}), $Ca_V1.2$ (α_{1C}), $Ca_V1.3$ (α_{1D}), and $Ca_V1.4$ (α_{1F}). The Ca_V2 subfamily includes $Ca_V2.1$ (α_{1A}), $Ca_V2.2$ (α_{1B}), and $Ca_V2.3$ (α_{1E}) which correspond to P/Q-type, N-type, and R-type Ca^{2+} currents, respectively. The Ca_V3 subfamily carries T-type Ca^{2+} currents, and the family members are $Ca_V3.1$ (α_{1G}), $Ca_V3.2$ (α_{1H}), and $Ca_V3.3$ (α_{1I}) [186]. The channel's auxiliary subunits are also organized into subfamilies, and these specifically affect membrane trafficking and expression of channels, voltage-dependence of channel opening, inactivation kinetics, and sensitivities to inhibitors, thus greatly expanding the number of different subtypes of VGCCs [185, 187]. In this section, we will discuss the contribution of each major type of VGCC to LTP in the spinal DH to various forms of pain in normal and pathological states.

4.1. L-Type VGCCs

4.1.1. Contribution to LTP. L-type VGCCs are widely expressed in the CNS [188], including CA1 of the hippocampus, the preeminent region for investigations of LTP. The dendritic localization of L-type VGCCs in the CA1 area [189] implies that their activation contributes to Ca^{2+} signals in dendritic spines, an important step for the induction of LTP [190]. Although induction of canonical LTP in CA1 relies upon Ca^{2+} flux through NMDA receptors on dendritic spines and subsequent activation of Ca^{2+}-dependent second messengers [70], several other forms of LTP in fact require activation of L-type VGCCs, and not NMDA receptors. In the hippocampal CA1 area, for example, HFS at 200 Hz [191], (higher frequency than the 100 Hz tetani typically employed for induction of NMDA receptor-dependent LTP) generates LTP that is insensitive to the NMDA receptor antagonist D-AP5 but is blocked by the L-type VGCC antagonist, nifedipine. While NMDA receptor-dependent LTP is inhibited by antagonists of serine-threonine kinases, the 200 Hz-induced, L-type VGCC-dependent LTP is blocked by antagonists of tyrosine kinases [192]. In addition, prolonged theta burst stimulation (TBS) in the CA1 area induces a form of LTP that is dependent upon L-type VGCCs [193]. L-type VGCC-dependent LTP can also be produced by application of the potassium channel blocker, tetraethylammonium (TEA) [194, 195]. Interestingly,

the mechanism of induction of this type of LTP partially overlaps that of NMDA receptor-dependent LTP, particularly in regard to the timing and intensity of postsynaptic Ca^{2+} signals [195]. Extracellular matrix molecules, such as hyaluronic acid and tenascin-R, are important in the development of L-type VGCC-dependent LTP induced by either TBS [196] or TEA [197]. As for Schaffer collateral-CA1 pyramidal neuron synapses, L-channel-dependent LTP has been described at mossy fiber synapses onto CA3 pyramidal neurons [198] and for thalamic inputs to amygdala [199]. Induction of NMDA receptor-independent, L-channel-dependent LTP is distinctive in its reliance upon such electrical phenomena as dendritic Ca^{2+} spikes [200–202].

In spinal DH, although L-type VGCCs are known to be expressed in soma, dendrites, and axon terminals of neurons [203, 204], it appears that L-channels do not induce LTP during 100 Hz repetitive stimulation [98]. L-type VGCCs contribute instead to an alternative form of LTP in spinal lamina I neurons, one that is induced by postsynaptic depolarization without presynaptic stimulation ("non-Hebbian" LTP) [205]. Gabapentin, which binds to the $\alpha_2\delta$ subunit of VGCCs and is used to relieve neuropathic pain, does not affect C fiber-mediated basal transmission or LTP induction but does reduce C fiber-mediated transmission during the maintenance phase of LTP [206]. Thus, postsynaptic Ca^{2+} influx through L-type VGCCs may be critical to induce or express LTP of excitatory synaptic transmission in certain normal and/or pathological states. More extensive investigation of distinct types of LTP induced under normal or neuropathological conditions is clearly needed to better understand the contribution of L-type VGCCs to synaptic plasticity and neuropathic pain of the spinal DH.

4.1.2. Contribution to Pain. Implication of L-type VGCCs in acute and chronic pain has been controversial. Some reports show that spinal administration of L-type VGCC blockers decreases pain sensitivity to acute innocuous or noxious stimuli [207, 208], but other work has found no effect of these blockers in the hot plate test [209] or in other tests using acute mechanical and thermal stimuli [210, 211]. Furthermore, in a chronic pain model of peripheral nerve injury, intrathecal administration of a high dose of the L-type VGCC blocker, diltiazem, has no effect on paw withdrawal in response to mechanical stimulation [212].

Recently, however, it has been found that prolonged intrathecal administration of the L-channel blocker nicardipine elevates mechanical threshold in a neuropathic pain model [213], indicating the involvement of L-type VGCCs in mechanical allodynia caused by peripheral nerve injury. Along the same lines, reduced expression of L-type VGCCs in spinal DH by antisense [213] or microRNA [214] technologies suppresses the hypersensitivity of DH neurons following peripheral nerve injury. Taken together, these findings indicate that L-type VGCCs can contribute to some components of acute or chronic pain behaviors produced by tissue damage, likely reflecting the contribution of L-type VGCCs to certain forms of LTP in the spinal DH.

4.2. P/Q-Type VGCCs

4.2.1. Contribution to LTP. P/Q-type VGCCs are expressed in a subpopulation of DRG neurons [203, 215, 216] that does not respond to capsaicin and rarely expresses substance P, a marker for small high-threshold primary afferent terminals [203]; P/Q channels thus play only a small role in the control of glutamate release from small diameter, peptidergic nociceptive primary afferent fibers. In addition, it has been suggested that P/Q-type VGCCs are expressed in fewer numbers of primary afferents than are N-type VGCCs [203, 217, 218]. In the spinal DH, P/Q-type VGCCs are expressed in the laminae II–VI [203] and are preferentially involved in inhibitory neurotransmission [219, 220], indicating a limited contribution of P/Q-type VGCCs to excitatory synaptic transmission in the spinal DH.

Blockers of P/Q-type VGCCs strongly suppress induction of LTP for C fiber-evoked field potentials [90], suggesting that induction of this form of spinal DH LTP may rely in part upon P/Q-type VGCCs [221]. Similarly, in visual cortical neurons, P/Q-type VGCCs have also been proposed to contribute to the induction of LTP at the inhibitory synapses [222].

4.2.2. Contribution to Pain. In accord with the minimal contribution of P/Q-type VGCCs to glutamate release from small-diameter, high-threshold primary afferents [203], intrathecal administration of the selective P/Q channel blocker, ω-agatoxin IVA, has little or no effect on C- or Aδ fiber-mediated nociceptive transmission [223] or in tests of mechanical and thermal thresholds in neuropathic pain models [212, 224]. However, development of hyperalgesia or pathological pain is prevented by intrathecal pretreatment with blockers of P/Q-type VGCCs [209–211], as well as in animals with either a genetic deficiency [225] or spontaneous mutation [226] in P/Q-type VGCCs. These observations correlate with studies of spinal LTP that indicate a critical role for P/Q-type VGCCs in the induction of LTP of C fiber-evoked field potentials [90]. Therefore, as for L-type VGCCs, P/Q-type VGCCs may also be involved in the development or regulation of certain forms of chronic pain.

4.3. N-Type VGCCs

4.3.1. Contribution to LTP. N-type VGCCs are expressed in dorsal root ganglia, as well as in primary afferent nerve terminals in the superficial area (laminae I-II) of DH [203, 217]. In accord with these findings, glutamatergic transmission between DRG and spinal DH neurons is blocked by ω-conotoxin GVIA, a selective blocker of N-type VGCCs [227]. Many of the presynaptic nerve terminals with N-type VGCC immunoreactivity also contain substance P, suggesting that N-type channels also support the release of substance P and CGRP from peptidergic, high-threshold C fibers in the spinal DH [228, 229].

As for other VGCCs, the involvement of N-type VGCCs in synaptic plasticity, particularly LTP, appears to be specific to the synaptic pathway and induction protocol. An early study showed that when a 100 Hz induction protocol is used, N-type VGCCs are not involved in LTP of

the hippocampal mossy fiber-mediated CA3 pathway [201]. However, at hippocampal CA3-to-CA1 Schaffer collateral synapses, when TBS or 200 Hz HFS is used to induce LTP, N-channel-mediated component of excitatory transmission can be identified after induction of LTP [230]. For perforant path synapses onto CA1 neurons, induction of LTP by 200 Hz HFS relies upon an increased contribution from presynaptic N-type VGCCs [231]. In the spinal DH, the N-channel blocker ω-conotoxin GVIA does not prevent induction of LTP of C fiber-field potentials, but this N channel antagonist inhibits synaptic transmission once LTP has been induced [90]. These results indicate that presynaptic N-type channels contribute to the maintenance phase of LTP in the spinal DH [231].

4.3.2. Contribution to Pain. Implication of N-type VGCCs in acute and chronic pain states correlates with the fact that N-type channels are predominantly expressed in DRG neurons, particularly small-diameter peptidergic DRG neurons and the fact that N channels may control release of neurotransmitters such as glutamate and substance P onto spinal DH neurons [228, 229]. Antagonists of N-type channels block the release of glutamate, substance P, and CGRP in the spinal DH [229, 232, 233], and in various acute, inflammatory, and neuropathic pain models, produce antinociceptive and analgesic effects [234, 235]. In addition, genetic ablation of $Ca_V2.2$ (α_{1B}), the pore-forming subunit of N-type channels, significantly reduces mechanical allodynia and thermal hyperalgesia in a neuropathic pain model with spinal nerve ligation [236]. Moreover, the antinociceptive effects of N-channel antagonists are enhanced in chronic pain states [237, 238], as N-type channels are upregulated after peripheral nerve injury [239]. Interestingly, these findings correlate with the observation that the block of N-channels inhibits synaptic transmission once LTP has been established [90].

Even though neuropathic pain can be reduced by blockers of N-type channels, and blockers such as ω-conotoxin MVIIA (SNX-111, ziconotide, or Prialt) have received approval by the FDA for the treatment of chronic pain; N-channel blockers are of limited use owing to side effects attributable to the fact that almost all of the presynaptic terminals in the brain express the N-type VGCCs. Thus, N-channel antagonists must be administered intrathecally, an invasive method used when other pain management options have failed. It may therefore prove useful to develop N-channel blockers that are either specific to particular $Ca_V1.2$ α_{1B} splice variants [240, 241] or that modulate N-type channel in selective neuronal subtypes [242].

4.4. T-Type VGCCs

4.4.1. Contribution to LTP. Although the lack of a specific antagonist prevents clean isolation of the contribution of T-type VGCCs to LTP, there is evidence indicating that T-type VGCCs are involved in the induction and/or expression of LTP in hippocampal CA1 [243] neurons and dentate gyrus [244]. The late phase of LTP in the CA1 area, which can be induced by 100 Hz HFS and is dependent on NMDA receptors, is not maintained (<120 min) in $Ca_V3.2$ T-type VGCC knockout mice [243]. LTP induction in dentate gyrus is sensitive to Ni^{2+}, a blocker of T-type as well as R-type VGCCs, and is dependent on the induction protocol: the Ni^{2+}-sensitive component of LTP can be induced by pairing of 1 Hz presynaptic stimulation with postsynaptic depolarization, but not by 100 Hz HFS [244]. T-type VGCCs may also be involved in LTP that is induced by TBS [245] or TEA [246] in the CA1 area, although L-channels have alternatively been reported to mediate TEA-induced LTP [195]. T-type VGCCs in CA1 are implicated in the enhancement of LTP, rather than its induction or expression, with the mechanism involving muscarinic acetylcholine receptors and phospholipase C-mediated K^+ channel inhibition [247].

T-type VGCCs are expressed in the superficial DH of the spinal cord, as well as in medium- and small-diameter DRG neurons [248], raising the possibility of both pre- and postsynaptic contributions to synaptic plasticity at primary afferent-DH neuron synapses. T-type Ca^{2+} currents have been reported in spinal DH neurons [223, 249–253]. In addition, the contribution of T-type Ca^{2+} channels to LTP of C fiber-initiated EPSCs in lamina I neurons that project to the PB area has been demonstrated [99], suggesting that T channels may play a significant role in amplification of pain signals via their contribution to spinal LTP. On the other hand, LFS-induced LTP of C fiber-EPSCs in PAG-projecting lamina I neurons [87] likely involves T-type VGCCs [254]. These results underscore the idea, once again, that spinal LTP is cell type-specific.

4.4.2. Contribution to Pain. The involvement of T-type VGCCs in pain is likely specific to T-channel subtypes. Nociceptive responses induced by nerve injury are decreased after knock down of $Ca_V3.2$, but not of $Ca_V3.1$ or $Ca_V3.3$ [255], presumably reflecting the abundance of $Ca_V3.2$ in DRG neurons [255–257] and indicating that the T-channel subtype involved in spinal LTP may be $Ca_V3.2$. In support of this interpretation, genetic knockout of $Ca_V3.2$ attenuates behavioral responses to noxious stimuli such as formalin [258]. In contrast, knockout of $Ca_V3.1$ causes hypersensitivity to noxious visceral stimuli, but this involves a supraspinal mechanism [259]. Therefore, although some nonselective T-type blockers such as ethosuximide or mibefradil can reverse both tactile hypersensitivity and thermal hyperalgesia in various pain models [260, 261], development of subtype-specific antagonists of T-type channels is desirable. In this regard, downregulation of T-type channel activity and of hyperalgesia by oxidizing agents [262, 263] or by lowering levels of the endogenous gasotransmitter hydrogen sulfide [264, 265] may prove useful as leads in developing novel subtype-selective T-channel drugs for the treatment of inflammatory or neuropathic pain.

4.5. R-Type VGCCs

4.5.1. Contribution to LTP. The involvement of R-type VGCCs in LTP can be difficult to isolate, in part because a commonly-used R-type channel blocker Ni^{2+} is also an

effective blocker of T-type channels. However, there is strong evidence that R-type channels support a presynaptic form of LTP found at parallel fiber synapses onto Purkinje cells in the cerebellum [266]. Because cerebellar granule cells (which give rise to parallel fibers) do not express T-type channels, in this system, the effects of Ni^{2+} block of Ca^{2+} current can be entirely attributed to antagonism of R-type channels. In comparison to N- and P/Q-channel-mediated Ca^{2+} influx at parallel fiber terminals, R-type channels contribute only modestly to bulk changes in intracellular Ca^{2+}, suggesting that R-channel Ca^{2+} microdomains in presynaptic terminals are important for the induction of parallel fiber LTP [266].

On the postsynaptic side, R-type VGCCs in CA1 pyramidal neurons contribute to Ca^{2+} influx evoked by TBS-triggered, back-propagating dendritic action potentials [267, 268]. In turn, R-channel Ca^{2+} influx in distal dendrites of CA1 pyramidal neurons helps generate plateau potentials that are critical for perforant path LTP [269].

Although R-type VGCCs are expressed in a subpopulation of DRG neurons [270], it is unclear whether the primary afferent terminals or spinal DH neurons bear R-type channels. In addition, whether R-type channels are involved in synaptic plasticity in the spinal DH remains to be determined.

4.5.2. Contribution to Pain. There is evidence that R-type VGCCs are involved in the transmission and processing of inflammatory and neuropathic pain information. SNX-482, an inhibitor of R-type VGCCs (and less potently of L-type channels) [271], decreases nociceptive responses during the second phase of the formalin test [217] and inhibits neuropathic pain behavior [272]. In addition, studies using $Ca_V2.3$ knockout mice suggest a contribution of R-type VGCCs to pain transmission [217, 273].

5. Concluding Remarks

Many studies have attempted to elucidate rules and signaling mechanisms for synaptic plasticity, particularly LTP, in the spinal DH. Together, these studies show that LTP in the spinal DH shares many features with LTP in the hippocampus and with "central sensitization" in the spinal DH during hyperalgesia [274]. In this review, we have considered how interrelationships between synaptic circuitry in the spinal DH, ionotropic glutamate receptors, voltage-gated Ca^{2+} channels, and induction/expression of LTP in the spinal DH are together involved in pain hypersensitivity.

Up to the present time, the complexity of synaptic circuitry in the spinal DH has hampered the understanding of LTP mechanisms in spinal DH. Generally, thorough classification of postsynaptic neurons and presynaptic fibers remains to be worked out. Although presynaptic fiber type can be identified during electrophysiological recording based upon conduction velocity and stimulus intensity, selective stimulation of a single class of primary afferent fiber remains challenging, because the range of stimulus intensities that activate C fibers overlaps the range of intensities that activates A fibers. Postsynaptically, in the spinal DH, although multiple morphological and electrophysiological criteria are available to distinguish neuronal subtypes, studying synaptic plasticity in a single type of spinal postsynaptic neuron has yet to be achieved, owing to inhomogeneities in physiological behavior, neurotransmitters, and cellular markers even within a group of neurons that carries out a similar function, such as the lamina I projection neurons [7].

In the future, progress in this field will likely rely upon studies that make use of powerful new experimental approaches, such as combining transgenic means to identify postsynaptic neurons [29, 32] and presynaptic fibers [25] with optogenetic tools to selectively activate specific fiber types [275]. This kind of approach will make it possible to study LTP at synapses between specific types of primary afferent fibers and spinal DH neurons or between specific spinal DH neurons, thereby facilitating the correlation between mechanisms of LTP and nociception in the spinal DH.

Acknowledgments

This work was supported by Kyungpook National University Research Fund, 2012, (2013, 2014) and US NIH Grant R01 HL088548.

References

[1] T. V. P. Bliss and T. Lomo, "Long lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path," *Journal of Physiology*, vol. 232, no. 2, pp. 331–356, 1973.

[2] M. Randić, M. C. Jiang, and R. Cerne, "Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord," *Journal of Neuroscience*, vol. 13, no. 12, pp. 5228–5241, 1993.

[3] B. Rexed, "The cytoarchitectonic organization of the spinal cord in the cat," *Journal of Comparative Neurology*, vol. 96, no. 3, pp. 414–495, 1952.

[4] C. Molander, Q. Xu, and G. Grant, "The cytoarchitectonic organization of the spinal cord in the rat. I. The lower thoracic and lumbosacral cord," *Journal of Comparative Neurology*, vol. 230, no. 1, pp. 133–141, 1984.

[5] J. M. A. Laird and F. Cervero, "A comparative study of the changes in receptive-field properties of multireceptive and nocireceptive rat dorsal horn neurons following noxious mechanical stimulation," *Journal of Neurophysiology*, vol. 62, no. 4, pp. 854–863, 1989.

[6] J. R. McClung and A. J. Castro, "Rexed's laminar scheme as it applies to the rat cervical spinal cord," *Experimental Neurology*, vol. 58, no. 1, pp. 145–148, 1978.

[7] A. J. Todd, "Neuronal circuitry for pain processing in the dorsal horn," *Nature Reviews Neuroscience*, vol. 11, no. 12, pp. 823–836, 2010.

[8] A. R. Light and E. R. Perl, "Reexamination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibers," *Journal of Comparative Neurology*, vol. 186, no. 2, pp. 117–131, 1979.

[9] A. R. Light and E. R. Perl, "Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers," *Journal of Comparative Neurology*, vol. 186, no. 2, pp. 133–150, 1979.

[10] C. LaMotte, "Distribution of the tract of Lissauer and the dorsal root fibers in the primate spinal cord," *Journal of Comparative Neurology*, vol. 172, no. 3, pp. 529–561, 1977.

[11] B. Lynn, "Effect of neonatal treatment with capsaicin on the numbers and properties of cutaneous afferent units from the hair skin of the rat," *Brain Research*, vol. 322, no. 2, pp. 255–260, 1984.

[12] B. Lynn and S. E. Carpenter, "Primary afferent units from the hairy skin of the rat hind limb," *Brain Research*, vol. 238, no. 1, pp. 29–43, 1982.

[13] H. J. Ralston and D. D. Ralston, "Identification of dorsal root synaptic terminals on monkey ventral horn cells by electron microscopic autoradiography," *Journal of Neurocytology*, vol. 8, no. 2, pp. 151–166, 1979.

[14] J. A. Beal and H. R. Bicknell, "Primary afferent distribution pattern in the marginal zone (lamina I) of adult monkey and cat lumbosacral spinal cord," *Journal of Comparative Neurology*, vol. 202, no. 2, pp. 255–263, 1981.

[15] J. I. Nagy and S. P. Hunt, "The termination of primary afferents within the rat dorsal horn: evidence for rearrangement following capsaicin treatment," *Journal of Comparative Neurology*, vol. 218, no. 2, pp. 145–158, 1983.

[16] S. Gobel, W. M. Falls, and E. Humphrey, "Morphology and synaptic connections of ultrafine primary axons in lamina I of the spinal dorsal horn: candidates for the terminal axonal arbors of primary neurons with unmyelinated (C) axons," *Journal of Neuroscience*, vol. 1, no. 10, pp. 1163–1179, 1981.

[17] S. N. Lawson, B. A. Crepps, and E. R. Perl, "Relationship of substance P to afferent characteristics of dorsal root ganglion neurones in guinea-pig," *Journal of Physiology*, vol. 505, no. 1, pp. 177–191, 1997.

[18] D. C. Molliver, D. E. Wright, M. L. Leitner et al., "IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life," *Neuron*, vol. 19, no. 4, pp. 849–861, 1997.

[19] G. J. Michael, S. Averill, A. Nitkunan et al., "Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord," *Journal of Neuroscience*, vol. 17, no. 21, pp. 8476–8490, 1997.

[20] J. D. Silverman and L. Kruger, "Selective neuronal glycoconjugate expression in sensory and autonomic ganglia: relation of lectin reactivity to peptide and enzyme markers," *Journal of Neurocytology*, vol. 19, no. 5, pp. 789–801, 1990.

[21] M. J. Zylka, F. L. Rice, and D. J. Anderson, "Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to Mrgprd," *Neuron*, vol. 45, no. 1, pp. 17–25, 2005.

[22] A. Guo, L. Vulchanova, J. Wang, X. Li, and R. Elde, "Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neliropeptides, the P2X3 purinoceptor and IB4 binding sites," *European Journal of Neuroscience*, vol. 11, no. 3, pp. 946–958, 1999.

[23] M. B. Gerke and M. B. Plenderleith, "Binding sites for the plant lectin *Bandeiraea simplicifolia* I-isolectin B4 are expressed by nociceptive primary sensory neurones," *Brain Research*, vol. 911, no. 1, pp. 101–104, 2001.

[24] D. J. Cavanaugh, H. Lee, L. Lo et al., "Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 22, pp. 9075–9080, 2009.

[25] L. Li, M. Rutlin, V. E. Abraira et al., "The functional organization of cutaneous low-threshold mechanosensory neurons," *Cell*, vol. 147, no. 7, pp. 1615–1627, 2011.

[26] R. Melzack and P. D. Wall, "Pain mechanisms: a new theory," *Science*, vol. 150, no. 3699, pp. 971–979, 1965.

[27] H. U. Zeilhofer, H. Wildner, and G. E. Yévenes, "Fast synaptic inhibition in spinal sensory processing and pain control," *Physiological Reviews*, vol. 92, no. 1, pp. 193–235, 2012.

[28] L. Cui, Y. R. Kim, H. Y. Kim et al., "Modulation of synaptic transmission from primary afferents to spinal substantia gelatinosa neurons by group III mGluRs in GAD65-EGFP transgenic mice," *Journal of Neurophysiology*, vol. 105, no. 3, pp. 1102–1111, 2011.

[29] B. Heinke, R. Ruscheweyh, L. Forsthuber, G. Wunderbaldinger, and J. Sandkühler, "Physiological, neurochemical and morphological properties of a subgroup of GABAergic spinal lamina II neurones identified by expression of green fluorescent protein in mice," *Journal of Physiology*, vol. 560, no. 1, pp. 249–266, 2004.

[30] A. J. Todd and A. C. Sullivan, "Light microscope study of the coexistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat," *Journal of Comparative Neurology*, vol. 296, no. 3, pp. 496–505, 1990.

[31] T. J. Grudt and E. R. Perl, "Correlations between neuronal morphology and electro-physiological features in the rodent superficial dorsal horn," *Journal of Physiology*, vol. 540, no. 1, pp. 189–207, 2002.

[32] A. W. Hantman, A. N. van den Pol, and E. R. Perl, "Morphological and physiological features of a set of spinal substantia gelatinosa neurons defined by green fluorescent protein expression," *Journal of Neuroscience*, vol. 24, no. 4, pp. 836–842, 2004.

[33] Y. Lu and E. R. Perl, "A specific inhibitory pathway between substantia gelatinosa neurons receiving direct C-fiber input," *Journal of Neuroscience*, vol. 23, no. 25, pp. 8752–8758, 2003.

[34] Y. Lu and E. R. Perl, "Modular organization of excitatory circuits between neurons of the spinal superficial dorsal horn (laminae I and II)," *Journal of Neuroscience*, vol. 25, no. 15, pp. 3900–3907, 2005.

[35] J. Zheng, Y. Lu, and E. R. Perl, "Inhibitory neurones of the spinal substantia gelatinosa mediate interaction of signals from primary afferents," *Journal of Physiology*, vol. 588, no. 12, pp. 2065–2075, 2010.

[36] T. Yasaka, G. Kato, H. Furue et al., "Cell-type-specific excitatory and inhibitory circuits involving primary afferents in the substantia gelatinosa of the rat spinal dorsal horn in vitro," *Journal of Physiology*, vol. 581, part 2, pp. 603–618, 2007.

[37] A. J. Todd and J. McKenzie, "GABA-immunoreactive neurons in the dorsal horn of the rat spinal cord," *Neuroscience*, vol. 31, no. 3, pp. 799–806, 1989.

[38] D. J. Maxwell, M. D. Belle, O. Cheunsuang, A. Stewart, and R. Morris, "Morphology of inhibitory and excitatory interneurons in superficial laminae of the rat dorsal horn," *Journal of Physiology*, vol. 584, part 2, pp. 521–533, 2007.

[39] E. Polgár, S. A. S. Shehab, C. Watt, and A. J. Todd, "GABAergic neurons that contain neuropeptide Y selectively target cells with the neurokinin I receptor in laminae III and IV of the rat spinal cord," *Journal of Neuroscience*, vol. 19, no. 7, pp. 2637–2646, 1999.

[40] E. Polgár, T. C. P. Sardella, M. Watanabe, and A. J. Todd, "Quantitative study of NPY-expressing GABAergic neurons and axons in rat spinal dorsal horn," *Journal of Comparative Neurology*, vol. 519, no. 6, pp. 1007–1023, 2011.

[41] Z. Puskár, E. Polgár, and A. J. Todd, "A population of large lamina I projection neurons with selective inhibitory input in rat spinal cord," *Neuroscience*, vol. 102, no. 1, pp. 167–176, 2001.

[42] S. F. Traynelis, L. P. Wollmuth, C. J. McBain et al., "Glutamate receptor ion channels: structure, regulation, and function," *Pharmacological Reviews*, vol. 62, no. 3, pp. 405–496, 2010.

[43] C. E. Jahr and T. M. Jessell, "Synaptic transmission between dorsal root ganglion and dorsal horn neurons in culture: antagonism of monosynaptic excitatory postsynaptic potentials and glutamate excitation by kynurenate," *Journal of Neuroscience*, vol. 5, no. 8, pp. 2281–2289, 1985.

[44] I. Kangrga and M. Randić, "Outflow of endogenous aspartate and glutamate from the rat spinal dorsal horn in vitro by activation of low- and high-threshold primary afferent fibers. Modulation by μ-opioids," *Brain Research*, vol. 553, no. 2, pp. 347–352, 1991.

[45] G. Gerber and M. Randić, "Excitatory amino acid-mediated components of synaptically evoked input from dorsal roots to deep dorsal horn neurons in the rat spinal cord slice," *Neuroscience Letters*, vol. 106, no. 1-2, pp. 211–219, 1989.

[46] M. Yoshimura and T. Jessell, "Amino acid-mediated EPSPs at primary afferent synapses with substantia gelatinosa neurones in the rat spinal cord," *Journal of Physiology*, vol. 430, pp. 315–335, 1990.

[47] P. Li, T. J. Wilding, S. J. Kim, A. A. Calejesan, J. E. Huettner, and M. Zhuo, "Kainate-receptor-mediated sensory synaptic transmission in mammalian spinal cord," *Nature*, vol. 397, no. 6715, pp. 161–164, 1999.

[48] D. H. Youn, N. Voitenko, G. Gerber, Y. K. Park, J. Galik, and M. Randić, "Altered long-term synaptic plasticity and kainate-induced Ca^{2+} transients in the substantia gelatinosa neurons in GLU_{K6}-deficient mice," *Molecular Brain Research*, vol. 142, no. 1, pp. 9–18, 2005.

[49] P. Li and M. Zhou, "Silent glutamatergic synapses and nociception in mammalian spinal cord," *Nature*, vol. 393, no. 6686, pp. 695–698, 1998.

[50] H. Baba, T. P. Doubell, K. A. Moore, and C. J. Woolf, "Silent NMDA receptor-mediated synapses are developmentally regulated in the dorsal horn of the rat spinal cord," *Journal of Neurophysiology*, vol. 83, no. 2, pp. 955–962, 2000.

[51] A. J. Todd, C. Watt, R. C. Spike, and W. Sieghart, "Colocalization of GABA, glycine, and their receptors at synapses in the rat spinal cord," *Journal of Neuroscience*, vol. 16, no. 3, pp. 974–982, 1996.

[52] N. Chéry and Y. de Koninck, "Junctional versus extrajunctional glycine and GABA(A) receptor-mediated IPSCs in identified lamina I neurons of the adult rat spinal cord," *Journal of Neuroscience*, vol. 19, no. 17, pp. 7342–7355, 1999.

[53] M. L. Baccei and M. Fitzgerald, "Development of GABAergic and glycinergic transmission in the neonatal rat dorsal horn," *Journal of Neuroscience*, vol. 24, no. 20, pp. 4749–4757, 2004.

[54] M. Yoshimura and S. Nishi, "Primary afferent-evoked glycine- and GABA-mediated IPSPs in substantia gelatinosa neurones in the rat spinal cord in vitro," *Journal of Physiology*, vol. 482, part 1, pp. 29–38, 1995.

[55] J. Lisman, R. C. Malenka, R. A. Nicoll, and R. Malinow, "Learning mechanisms: the case for CaM-KII," *Science*, vol. 276, no. 5321, pp. 2001–2002, 1997.

[56] A. Barria, D. Muller, V. Derkach, L. C. Griffith, and T. R. Soderling, "Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation," *Science*, vol. 276, no. 5321, pp. 2042–2045, 1997.

[57] H. K. Lee, M. Barbarosie, K. Kameyama, M. F. Bear, and R. L. Huganir, "Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity," *Nature*, vol. 405, no. 6789, pp. 955–959, 2000.

[58] H. K. Lee, K. Takamiya, J. S. Han et al., "Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory," *Cell*, vol. 112, no. 5, pp. 631–643, 2003.

[59] J. Lisman, R. Yasuda, and S. Raghavachari, "Mechanisms of CaMKII action in long-term potentiation," *Nature Reviews Neuroscience*, vol. 13, no. 3, pp. 169–182, 2012.

[60] T. G. Banke, D. Bowie, H. K. Lee, R. L. Huganir, A. Schousboe, and S. F. Traynelis, "Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase," *Journal of Neuroscience*, vol. 20, no. 1, pp. 89–102, 2000.

[61] T. A. Benke, A. Lüthi, J. T. R. Isaac, and G. L. Collingridge, "Modulation of ampa receptor unitary conductance by synaptic activity," *Nature*, vol. 393, no. 6687, pp. 793–797, 1998.

[62] S. H. Shi, Y. Hayashi, R. S. Petralia et al., "Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation," *Science*, vol. 284, no. 5412, pp. 1811–1816, 1999.

[63] S. Q. J. Liu and S. G. Cull-Candy, "Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype," *Nature*, vol. 405, no. 6785, pp. 454–458, 2000.

[64] R. Malinow and R. C. Malenka, "AMPA receptor trafficking and synaptic plasticity," *Annual Review of Neuroscience*, vol. 25, pp. 103–126, 2002.

[65] P. E. Schulz, "Long-term potentiation involves increases in the probability of neurotransmitter release," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 11, pp. 5888–5893, 1997.

[66] D. M. Kullmann and R. A. Nicoll, "Long-term potentiation is associated with increases in quantal content and quantal amplitude," *Nature*, vol. 357, no. 6375, pp. 240–244, 1992.

[67] J. Lisman and S. Raghavachari, "A unified model of the presynaptic and postsynaptic changes during LTP at CA1 synapses," *Science's STKE*, vol. 2006, no. 356, p. re11, 2006.

[68] T. V. Bliss and G. L. Collingridge, "Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide," *Molecular Brain*, vol. 6, article 5, 2013.

[69] T. V. P. Bliss and G. L. Collingridge, "A synaptic model of memory: long-term potentiation in the hippocampus," *Nature*, vol. 361, no. 6407, pp. 31–39, 1993.

[70] R. C. Malenka and R. A. Nicoll, "Long-term potentiation—a decade of progress?" *Science*, vol. 285, no. 5435, pp. 1870–1874, 1999.

[71] M. A. Lynch, "Long-Term potentiation and memory," *Physiological Reviews*, vol. 84, no. 1, pp. 87–136, 2004.

[72] J. R. Whitlock, A. J. Heynen, M. G. Shuler, and M. F. Bear, "Learning induces long-term potentiation in the hippocampus," *Science*, vol. 313, no. 5790, pp. 1093–1097, 2006.

[73] X. G. Liu and J. Sandkühler, "Long-term potentiation of C-fiber-evoked potentials in the rat spinal dorsal horn is prevented by spinal N-methyl-D-aspartic acid receptor blockage," *Neuroscience Letters*, vol. 191, no. 1-2, pp. 43–46, 1995.

[74] M. Randić, "Plasticity of excitatory synaptic transmission in the spinal cord dorsal horn," *Progress in Brain Research*, vol. 113, pp. 463–506, 1996.

[75] X. G. Liu and J. Sandkühler, "Activation of spinal N-methyl-D-aspartate or neurokinin receptors induces long-term potentiation of spinal C-fibre-evoked potentials," *Neuroscience*, vol. 86, no. 4, pp. 1209–1216, 1998.

[76] X. G. Liu and J. Sandkühler, "Characterization of long-term potentiation of C-fiber-evoked potentials in spinal dorsal horn of adult rat: essential role of NK1 and NK2 receptors," *Journal of Neurophysiology*, vol. 78, no. 4, pp. 1973–1982, 1997.

[77] J. J. Azkue, X. G. Liu, M. Zimmermann, and J. Sandkühler, "Induction of long-term potentiation of C fibre-evoked spinal field potentials requires recruitment of group I, but not group II/III metabotropic glutamate receptors," *Pain*, vol. 106, no. 3, pp. 373–379, 2003.

[78] G. W. Terman, C. L. Eastman, and C. Chavkin, "Mu opiates inhibit long-term potentiation induction in the spinal cord slice," *Journal of Neurophysiology*, vol. 85, no. 2, pp. 485–494, 2001.

[79] S. M. Dudek and M. F. Bear, "Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 10, pp. 4363–4367, 1992.

[80] A. Kemp and D. Manahan-Vaughan, "Hippocampal long-term depression: master or minion in declarative memory processes?" *Trends in Neurosciences*, vol. 30, no. 3, pp. 111–118, 2007.

[81] P. V. Massey and Z. I. Bashir, "Long-term depression: multiple forms and implications for brain function," *Trends in Neurosciences*, vol. 30, no. 4, pp. 176–184, 2007.

[82] M. Ito, "Cerebellar long-term depression: characterization, signal transduction, and functional roles," *Physiological Reviews*, vol. 81, no. 3, pp. 1143–1195, 2001.

[83] G. Cheng and M. Randić, "Involvement of intracellular calcium and protein phosphatases in long-term depression of A-fiber-mediated primary afferent neurotransmission," *Developmental Brain Research*, vol. 144, no. 1, pp. 73–82, 2003.

[84] J. Sandkühler, J. G. Chen, G. Cheng, and M. Randić, "Low-frequency stimulation of afferent aδ-fibers induces long-term depression at primary afferent synapses with substantia gelatinosa neurons in the rat," *Journal of Neuroscience*, vol. 17, no. 16, pp. 6483–6491, 1997.

[85] J. Sandkühler, "Models and mechanisms of hyperalgesia and allodynia," *Physiological Reviews*, vol. 89, no. 2, pp. 707–758, 2009.

[86] R. Ruscheweyh, O. Wilder-Smith, R. Drdla, X. G. Liu, and J. Sandkühler, "Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy," *Molecular Pain*, vol. 7, article 20, 2011.

[87] H. Ikeda, J. Stark, H. Fischer et al., "Synaptic amplifier of inflammatory pain in the spinal dorsal horn," *Science*, vol. 312, no. 5780, pp. 1659–1662, 2006.

[88] R. Drdla-Schutting, J. Benrath, G. Wunderbaldinger, and J. Sandkühler, "Erasure of a spinal memory trace of pain by a brief, high-dose opioid administration," *Science*, vol. 335, no. 6065, pp. 235–238, 2012.

[89] J. Sandkühler and X. Liu, "Induction of long-term potentiation at spinal synapses by noxious stimulation or nerve injury," *European Journal of Neuroscience*, vol. 10, no. 7, pp. 2476–2480, 1998.

[90] S. Ohnami, M. Tanabe, S. Shinohara, K. Takasu, A. Kato, and H. Ono, "Role of voltage-dependent calcium channel subtypes in spinal long-term potentiation of C-fiber-evoked field potentials," *Pain*, vol. 152, no. 3, pp. 623–631, 2011.

[91] C. K. Park, N. Lü, Z. Z. Xu, T. Liu, C. N. Serhan, and R. R. Ji, "Resolving TRPV1- and TNF-α-mediated spinal cord synaptic plasticity and inflammatory pain with neuroprotectin D1," *Journal of Neuroscience*, vol. 31, no. 42, pp. 15072–15085, 2011.

[92] W. T. Liu, H. C. Li, X. S. Song, Z. J. Huang, and X. J. Song, "EphB receptor signaling in mouse spinal cord contributes to physical dependence on morphine," *FASEB Journal*, vol. 23, no. 1, pp. 90–98, 2009.

[93] C. Luo, V. Gangadharan, K. K. Bali et al., "Presynaptically localized cyclic GMP-dependent protein kinase 1 is a key determinant of spinal synaptic potentiation and pain hypersensitivity," *PLoS Biology*, vol. 10, no. 3, Article ID e1001283, 2012.

[94] L. Z. Cheng, N. Lü, Y. Q. Zhang, and Z. Q. Zhao, "Ryanodine receptors contribute to the induction of nociceptive input-evoked long-term potentiation in the rat spinal cord slice," *Molecular Pain*, vol. 6, article 1, 2010.

[95] X. C. Zhang, Y. Q. Zhang, and Z. Q. Zhao, "Involvement of nitric oxide in long-term potentiation of spinal nociceptive responses in rats," *NeuroReport*, vol. 16, no. 11, pp. 1197–1201, 2005.

[96] X. C. Zhang, Y. Q. Zhang, and Z. Q. Zhao, "Different roles of two nitric oxide activated pathways in spinal long-term potentiation of C-fiber-evoked field potentials," *Neuropharmacology*, vol. 50, no. 6, pp. 748–754, 2006.

[97] L. M. Pedersen, L. M. Jacobsen, S. Mollerup, and J. Gjerstad, "Spinal cord long-term potentiation (LTP) is associated with increased dorsal horn gene expression of IL-1β, GDNF and iNOS," *European Journal of Pain*, vol. 14, no. 3, pp. 255–260, 2010.

[98] D. H. Youn, G. Royle, M. Kolaj, B. Vissel, and M. Randić, "Enhanced LTP of primary afferent neurotransmission in AMPA receptor GluR2-deficient mice," *Pain*, vol. 136, no. 1-2, pp. 158–167, 2008.

[99] H. Ikeda, B. Heinke, R. Ruscheweyh, and J. Sandkühler, "Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia," *Science*, vol. 299, no. 5610, pp. 1237–1240, 2003.

[100] H. Y. Zhou, S. R. Chen, H. Chen, and H. L. Pan, "Opioid-induced long-term potentiation in the spinal cord is a presynaptic event," *Journal of Neuroscience*, vol. 30, no. 12, pp. 4460–4466, 2010.

[101] G. Q. Bi and M. M. Poo, "Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type," *Journal of Neuroscience*, vol. 18, no. 24, pp. 10464–10472, 1998.

[102] J. M. Montgomery, P. Pavlidis, and D. V. Madison, "Pair recordings reveal all-silent synaptic connections and the postsynaptic expression of long-term potentiation," *Neuron*, vol. 29, no. 3, pp. 691–701, 2001.

[103] S. J. Jung, S. J. Kim, Y. K. Park, S. B. Oh, K. Cho, and J. Kim, "Group I mGluR regulates the polarity of spike-timing dependent plasticity in substantia gelatinosa neurons," *Biochemical and Biophysical Research Communications*, vol. 347, no. 2, pp. 509–516, 2006.

[104] F. Wei, K. I. Vadakkan, H. Toyoda et al., "Calcium calmodulin-stimulated adenylyl cyclases contribute to activation of extracellular signal-regulated kinase in spinal dorsal horn neurons in adult rats and mice," *Journal of Neuroscience*, vol. 26, no. 3, pp. 851–861, 2006.

[105] R. Dingledine, K. Borges, D. Bowie, and S. F. Traynelis, "The glutamate receptor ion channels," *Pharmacological Reviews*, vol. 51, no. 1, pp. 7–61, 1999.

[106] M. Hollmann and S. Heinemann, "Cloned glutamate receptors," *Annual Review of Neuroscience*, vol. 17, pp. 31–108, 1994.

[107] A. L. Mammen, K. Kameyama, K. W. Roche, and R. L. Huganir, "Phosphorylation of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II," *The Journal of Biological Chemistry*, vol. 272, no. 51, pp. 32528–32533, 1997.

[108] K. W. Roche, R. J. O'Brien, A. L. Mammen, J. Bernhardt, and R. L. Huganir, "Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit," *Neuron*, vol. 16, no. 6, pp. 1179–1188, 1996.

[109] Y. Hayashi, S. H. Shi, J. A. Esteban, A. Piccini, J. C. Poncer, and R. Malinow, "Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction," *Science*, vol. 287, no. 5461, pp. 2262–2267, 2000.

[110] S. H. Shi, Y. Hayashi, J. A. Esteban, and R. Malinow, "Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons," *Cell*, vol. 105, no. 3, pp. 331–343, 2001.

[111] D. Zamanillo, R. Sprengel, O. Hvalby et al., "Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning," *Science*, vol. 284, no. 5421, pp. 1805–1811, 1999.

[112] K. Plant, K. A. Pelkey, Z. A. Bortolotto et al., "Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation," *Nature Neuroscience*, vol. 9, no. 5, pp. 602–604, 2006.

[113] Z. Jia, N. Agopyan, P. Miu et al., "Enhanced LTP in mice deficient in the AMPA receptor GluR2," *Neuron*, vol. 17, no. 5, pp. 945–956, 1996.

[114] Y. Meng, Y. Zhang, and Z. Jia, "Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3," *Neuron*, vol. 39, no. 1, pp. 163–176, 2003.

[115] S. Tomita, H. Adesnik, M. Sekiguchi et al., "Stargazin modulates AMPA receptor gating and trafficking by distinct domains," *Nature*, vol. 435, no. 7045, pp. 1052–1058, 2005.

[116] S. Tomita, V. Stein, T. J. Stocker, R. A. Nicoll, and D. S. Bredt, "Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs," *Neuron*, vol. 45, no. 2, pp. 269–277, 2005.

[117] S. Tomita, L. Chen, Y. Kawasaki et al., "Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins," *Journal of Cell Biology*, vol. 161, no. 4, pp. 805–816, 2003.

[118] A. D. Milstein and R. A. Nicoll, "TARP modulation of synaptic AMPA receptor trafficking and gating depends on multiple intracellular domains," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 27, pp. 11348–11351, 2009.

[119] A. J. Granger, Y. Shi, W. Lu, M. Cerpas, and R. A. Nicoll, "LTP requires a reserve pool of glutamate receptors independent of subunit type," *Nature*, vol. 493, no. 7433, pp. 495–500, 2013.

[120] R. C. Spike, D. J. Maxwell, and A. J. Todd, "GluR1 and GluR2/3 subunits of the AMPA-type glutamate receptor are associated with particular types of neurone in laminae I–III of the spinal dorsal horn of the rat," *European Journal of Neuroscience*, vol. 10, no. 1, pp. 324–333, 1998.

[121] H. S. Engelman, T. B. Allen, and A. B. MacDermott, "The distribution of neurons expressing calcium-permeable AMPA receptors in the superficial laminae of the spinal cord dorsal horn," *Journal of Neuroscience*, vol. 19, no. 6, pp. 2081–2089, 1999.

[122] J. G. Gu, C. Albuquerque, C. J. Lee, and A. B. MacDermott, "Synaptic strengthening through activation of Ca^{2+}-permeable AMPA receptors," *Nature*, vol. 381, no. 6585, pp. 793–796, 1996.

[123] A. Popratiloff, R. J. Weinberg, and A. Rustioni, "AMPA receptor subunits underlying terminals of fine-caliber primary afferent fibers," *Journal of Neuroscience*, vol. 16, no. 10, pp. 3363–3372, 1996.

[124] H. Furukawa, S. K. Singh, R. Mancusso, and E. Gouaux, "Subunit arrangement and function in NMDA receptors," *Nature*, vol. 438, no. 7065, pp. 185–192, 2005.

[125] H. Furukawa and E. Gouaux, "Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core," *The EMBO Journal*, vol. 22, no. 12, pp. 2873–2885, 2003.

[126] Y. Yao, C. B. Harrison, P. L. Freddolino, K. Schulten, and M. L. Mayer, "Molecular mechanism of ligand recognition by NR3 subtype glutamate receptors," *The EMBO Journal*, vol. 27, no. 15, pp. 2158–2170, 2008.

[127] A. B. MacDermott, M. L. Mayer, G. L. Westbrook, S. J. Smith, and J. L. Barker, "NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones," *Nature*, vol. 321, no. 6069, pp. 519–522, 1986.

[128] M. L. Mayer, G. L. Westbrook, and P. B. Buthrie, "Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones," *Nature*, vol. 309, no. 5965, pp. 261–263, 1984.

[129] R. A. J. Lester, J. D. Clements, G. L. Westbrook, and C. E. Jahr, "Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents," *Nature*, vol. 346, no. 6284, pp. 565–567, 1990.

[130] S. Cull-Candy, S. Brickley, and M. Farrant, "NMDA receptor subunits: diversity, development and disease," *Current Opinion in Neurobiology*, vol. 11, no. 3, pp. 327–335, 2001.

[131] H. Monyer, N. Burnashev, D. J. Laurie, B. Sakmann, and P. H. Seeburg, "Developmental and regional expression in the rat brain and functional properties of four NMDA receptors," *Neuron*, vol. 12, no. 3, pp. 529–540, 1994.

[132] D. J. A. Wyllie, P. Béhé, and D. Colquhoun, "Single-channel activations and concentration jumps: comparison of recombinant NR1a/NR2A and NR1a/NR2D NMDA receptors," *Journal of Physiology*, vol. 510, part 1, pp. 1–18, 1998.

[133] S. Vicini, J. F. Wang, J. H. Li et al., "Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors," *Journal of Neurophysiology*, vol. 79, no. 2, pp. 555–566, 1998.

[134] S. Das, Y. F. Sasaki, T. Rothe et al., "Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A," *Nature*, vol. 393, no. 6683, pp. 377–381, 1998.

[135] I. Pérez-Otaño, C. T. Schulteis, A. Contractor et al., "Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors," *Journal of Neuroscience*, vol. 21, no. 4, pp. 1228–1237, 2001.

[136] J. E. Chatterton, M. Awobuluyi, L. S. Premkumar et al., "Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits," *Nature*, vol. 415, no. 6873, pp. 793–798, 2002.

[137] J. F. MacDonald, M. F. Jackson, and M. A. Beazely, "Hippocampal long-term synaptic plasticity and signal amplification of NMDA receptors," *Critical Reviews in Neurobiology*, vol. 18, no. 1-2, pp. 71–84, 2006.

[138] G. Köhr, V. Jensen, H. J. Koester et al., "Intracellular domains of NMDA receptor subtypes are determinants for long-term potentiation induction," *Journal of Neuroscience*, vol. 23, no. 34, pp. 10791–10799, 2003.

[139] T. E. Bartlett, N. J. Bannister, V. J. Collett et al., "Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus," *Neuropharmacology*, vol. 52, no. 1, pp. 60–70, 2007.

[140] Y. P. Tang, E. Shimizu, G. R. Dube et al., "Genetic enhancement of learning and memory in mice," *Nature*, vol. 401, no. 6748, pp. 63–69, 1999.

[141] K. Erreger, S. M. Dravid, T. G. Banke, D. J. A. Wyllie, and S. F. Traynelis, "Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles," *Journal of Physiology*, vol. 563, no. 2, pp. 345–358, 2005.

[142] H. H. Cheung and J. W. Gurd, "Tyrosine phosphorylation of the N-methyl-D-aspartate receptor by exogenous and postsynaptic density-associated Src-family kinases," *Journal of Neurochemistry*, vol. 78, no. 3, pp. 524–534, 2001.

[143] T. Nakazawa, S. Komai, T. Tezuka et al., "Characterization of Fyn-mediated tyrosine phosphorylation sites on GluRε2 (NR2B) subunit of the N-methyl-D-aspartate receptor," *The Journal of Biological Chemistry*, vol. 276, no. 1, pp. 693–699, 2001.

[144] D. Ng, G. M. Pitcher, R. K. Szilard et al., "Neto1 is a novel CUB-domain NMDA receptor-interacting protein required for synaptic plasticity and learning," *PLoS Biology*, vol. 7, no. 2, article e41, 2009.

[145] A. Barria and R. Malinow, "NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII," *Neuron*, vol. 48, no. 2, pp. 289–301, 2005.

[146] A. J. Scheetz, A. C. Nairn, and M. Constantine-Paton, "NMDA receptor-mediated control of protein synthesis at developing synapses," *Nature Neuroscience*, vol. 3, no. 3, pp. 211–216, 2000.

[147] R. Gong, S. P. Chang, N. R. Abbassi, and S. J. Tang, "Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons," *The Journal of Biological Chemistry*, vol. 281, no. 27, pp. 18802–18815, 2006.

[148] D. H. Tran, R. Gong, and S. J. Tang, "Differential roles of NR2A and NR2B subtypes in NMDA receptor-dependent protein synthesis in dendrites," *Neuropharmacology*, vol. 53, no. 2, pp. 252–256, 2007.

[149] K. D. Bradshaw, N. J. Emptage, and T. V. P. Bliss, "A role for dendritic protein synthesis in hippocampal late LTP," *European Journal of Neuroscience*, vol. 18, no. 11, pp. 3150–3152, 2003.

[150] T. R. Tölle, A. Berthele, W. Zieglgänsberger, P. H. Seeburg, and W. Wisden, "The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray," *Journal of Neuroscience*, vol. 13, no. 12, pp. 5009–5028, 1993.

[151] J. M. Luque, Z. Bleuel, P. Malherbe, and J. G. Richards, "Alternatively spliced isoforms of the N-methyl-D-aspartate receptor subunit 1 are differentially distributed within the rat spinal cord," *Neuroscience*, vol. 63, no. 3, pp. 629–635, 1994.

[152] S. Boyce, A. Wyatt, J. K. Webb et al., "Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn," *Neuropharmacology*, vol. 38, no. 5, pp. 611–623, 1999.

[153] G. G. Nagy, M. Watanabe, M. Fukaya, and A. J. Todd, "Synaptic distribution of the NR1, NR2A and NR2B subunits of the N-methyl-D-aspartate receptor in the rat lumbar spinal cord revealed with an antigen-unmasking technique," *European Journal of Neuroscience*, vol. 20, no. 12, pp. 3301–3312, 2004.

[154] H. Shiokawa, E. J. Kaftan, A. B. MacDermott, and C. K. Tong, "NR2 subunits and NMDA receptors on lamina II inhibitory and excitatory interneurons of the mouse dorsal horn," *Molecular Pain*, vol. 6, article 26, 2010.

[155] A. Momiyama, "Distinct synaptic and extrasynaptic NMDA receptors identified in dorsal horn neurones of the adult rat spinal cord," *Journal of Physiology*, vol. 523, part 3, pp. 621–628, 2000.

[156] J. Sandkühler, "Understanding LTP in pain pathways," *Molecular Pain*, vol. 3, article 9, 2007.

[157] S. K. Georgiev, H. Furue, H. Baba, and T. Kohno, "Xenon inhibits excitatory but not inhibitory transmission in rat spinal cord dorsal horn neurons," *Molecular Pain*, vol. 6, article 25, 2010.

[158] J. Benrath, C. Kempf, M. Georgieff, and J. Sandkühler, "Xenon blocks the induction of synaptic long-term potentiation in pain pathways in the rat spinal cord in vivo," *Anesthesia and Analgesia*, vol. 104, no. 1, pp. 106–111, 2007.

[159] H. Y. Kim, K. Y. Lee, Y. Lu et al., "Mitochondrial Ca^{2+} uptake is essential for synaptic plasticity in pain," *Journal of Neuroscience*, vol. 31, no. 36, pp. 12982–12991, 2011.

[160] J. Lerma, A. V. Paternain, A. Rodríguez-Moreno, and J. C. López-Garciáa, "Molecular physiology of kainate receptors," *Physiological Reviews*, vol. 81, no. 3, pp. 971–998, 2001.

[161] B. Bettler, J. Egebjerg, G. Sharma et al., "Cloning of a putative glutamate receptor: a low affinity kainate-binding subunit," *Neuron*, vol. 8, no. 2, pp. 257–265, 1992.

[162] P. Werner, M. Voigt, K. Keinanen, W. Wisden, and P. H. Seeburg, "Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells," *Nature*, vol. 351, no. 6329, pp. 742–744, 1991.

[163] A. Herb, N. Burnashev, P. Werner, B. Sakmann, W. Wisden, and P. H. Seeburg, "The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits," *Neuron*, vol. 8, no. 4, pp. 775–785, 1992.

[164] B. Sommer, N. Burnashev, T. A. Verdoorn, K. Keinanen, B. Sakmann, and P. H. Seeburg, "A glutamate receptor channel with high affinity for domoate and kainate," *The EMBO Journal*, vol. 11, no. 4, pp. 1651–1656, 1992.

[165] H. H. Schiffer, G. T. Swanson, and S. F. Heinemann, "Rat GluR7 and a carboxy-terminal splice variant, GluR7b, are functional kainate receptor subunits with a low sensitivity to glutamate," *Neuron*, vol. 19, no. 5, pp. 1141–1146, 1997.

[166] P. Gregor, B. F. O'Hara, X. Yang, and G. R. Uhl, "Expression and novel subunit isoforms of glutamate receptor genes GluR5 and GluR6," *NeuroReport*, vol. 4, no. 12, pp. 1343–1346, 1993.

[167] N. Burnashev, Z. Zhou, E. Neher, and B. Sakmann, "Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes," *Journal of Physiology*, vol. 485, part 2, pp. 403–418, 1995.

[168] J. Egebjerg and S. F. Heinemann, "Ca^{2+} permeability of unedited and edited versions of the kainate selective glutamate receptor GluR6," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 2, pp. 755–759, 1993.

[169] N. Burnashev, A. Villarroel, and B. Sakmann, "Dimensions and ion selectivity of recombinant AMPA and kainate receptor channels and their dependence on Q/R site residues," *Journal of Physiology*, vol. 496, part 1, pp. 165–173, 1996.

[170] A. Sailer, G. T. Swanson, I. Pérez-Otaño et al., "Generation and analysis of GluR5(Q636R) kainate receptor mutant mice," *Journal of Neuroscience*, vol. 19, no. 20, pp. 8757–8764, 1999.

[171] M. Kohler, N. Burnashev, B. Sakmann, and P. H. Seeburg, "Determinants of Ca^{2+} permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity by RNA editing," *Neuron*, vol. 10, no. 3, pp. 491–500, 1993.

[172] J. Lerma, "Roles and rules of kainate receptors in synaptic transmission," *Nature Reviews Neuroscience*, vol. 4, no. 6, pp. 481–495, 2003.

[173] Z. A. Bortolotto, V. R. J. Clarke, C. M. Delany et al., "Kainate receptors are involved in synaptic plasticity," *Nature*, vol. 402, no. 6759, pp. 297–301, 1999.

[174] S. E. Lauri, Z. A. Bortolotto, R. Nistico et al., "A role for Ca^{2+} stores in kainate receptor-dependent synaptic facilitation and LTP at mossy fiber synapses in the hippocampus," *Neuron*, vol. 39, no. 2, pp. 327–341, 2003.

[175] M. G. Weisskopf and R. A. Nicoll, "Presynaptic changes during mossy fibre LTP revealed by NMDA receptor-mediated synaptic responses," *Nature*, vol. 376, no. 6537, pp. 256–259, 1995.

[176] M. F. Yeckel, A. Kapur, and D. Johnston, "Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism," *Nature Neuroscience*, vol. 2, no. 7, pp. 625–633, 1999.

[177] A. Contractor, G. Swanson, and S. F. Heinemann, "Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus," *Neuron*, vol. 29, no. 1, pp. 209–216, 2001.

[178] S. J. Hwang, S. Pagliardini, A. Rustioni, and J. G. Valtschanoff, "Presynaptic kainate receptors in primary afferents to the superficial laminae of the rat spinal cord," *Journal of Comparative Neurology*, vol. 436, no. 3, pp. 275–289, 2001.

[179] G. A. Kerchner, T. J. Wilding, J. E. Huettner, and M. Zhuo, "Kainate receptor subunits underlying presynaptic regulation of transmitter release in the dorsal horn," *Journal of Neuroscience*, vol. 22, no. 18, pp. 8010–8017, 2002.

[180] G. A. Kerchner, T. J. Wilding, P. Li, M. Zhuo, and J. E. Huettner, "Presynaptic kainate receptors regulate spinal sensory transmission," *Journal of Neuroscience*, vol. 21, no. 1, pp. 59–66, 2001.

[181] K. M. Partin, D. K. Patneau, C. A. Winters, M. L. Mayer, and A. Buonanno, "Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concanavalin A," *Neuron*, vol. 11, no. 6, pp. 1069–1082, 1993.

[182] R. S. Petralia, Y. X. Wang, and R. J. Wenthold, "Histological and ultrastructural localization of the kainate receptor subunits, KA2 and GluR6/7, in the rat nervous system using selective antipeptide antibodies," *Journal of Comparative Neurology*, vol. 349, no. 1, pp. 85–110, 1994.

[183] J. E. Huettner, "Glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A," *Neuron*, vol. 5, no. 3, pp. 255–266, 1990.

[184] D. H. Youn and M. Randić, "Modulation of excitatory synaptic transmission in the spinal substantia gelatinosa of mice deficient in the kainate receptor GluR5 and/or GluR6 subunit," *Journal of Physiology*, vol. 555, part 3, pp. 683–698, 2004.

[185] W. A. Catterall, "Structure and regulation of voltage-gated Ca^{2+} channels," *Annual Review of Cell and Developmental Biology*, vol. 16, pp. 521–555, 2000.

[186] W. A. Catterall, E. Perez-Reyes, T. P. Snutch, and J. Striessnig, "International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels," *Pharmacological Reviews*, vol. 57, no. 4, pp. 411–425, 2005.

[187] Z. Buraei and J. Yang, "The β subunit of voltage-gated Ca^{2+} channels," *Physiological Reviews*, vol. 90, no. 4, pp. 1461–1506, 2010.

[188] T. P. Snutch, W. J. Tomlinson, J. P. Leonard, and M. M. Gilbert, "Distinct calcium channels are generated by alternative splicing and are differentially expressed in the mammalian CNS," *Neuron*, vol. 7, no. 1, pp. 45–57, 1991.

[189] R. E. Westenbroek, M. K. Ahlijanian, and W. A. Catterall, "Clustering of L-type Ca^{2+} channels at the base of major dendrites in hippocampal pyramidal neurons," *Nature*, vol. 347, no. 6290, pp. 281–284, 1990.

[190] S. J. R. Lee, Y. Escobedo-Lozoya, E. M. Szatmari, and R. Yasuda, "Activation of CaMKII in single dendritic spines during long-term potentiation," *Nature*, vol. 458, no. 7236, pp. 299–304, 2009.

[191] L. M. Grover and T. J. Teyler, "Two components of long-term potentiation induced by different patterns of afferent activation," *Nature*, vol. 347, no. 6292, pp. 477–479, 1990.

[192] I. Cavus and T. Teyler, "Two forms of long-term potentiation in area CA1 activate different signal transduction cascades," *Journal of Neurophysiology*, vol. 76, no. 5, pp. 3038–3047, 1996.

[193] S. L. Morgan and T. J. Teyler, "Electrical stimuli patterned after the theta-rhythm induce multiple forms of LTP," *Journal of Neurophysiology*, vol. 86, no. 3, pp. 1289–1296, 2001.

[194] L. Aniksztejn and Y. Ben-Ari, "Novel form of long-term potentiation produced by a K^+ channel blocker in the hippocampus," *Nature*, vol. 349, no. 6304, pp. 67–69, 1991.

[195] Y. Y. Huang and R. C. Malenka, "Examination of TEA-induced synaptic enhancement in area CA1 of the hippocampus: the role of voltage-dependent Ca^{2+} channels in the induction of LTP," *Journal of Neuroscience*, vol. 13, no. 2, pp. 568–576, 1993.

[196] G. Kochlamazashvili, C. Henneberger, O. Bukalo et al., "The extracellular matrix molecule hyaluronic acid regulates hippocampal synaptic plasticity by modulating postsynaptic L-type Ca^{2+} channels," *Neuron*, vol. 67, no. 1, pp. 116–128, 2010.

[197] M. R. Evers, B. Salmen, O. Bukalo et al., "Impairment of L-type Ca^{2+} channel-dependent forms of hippocampal synaptic plasticity in mice deficient in the extracellular matrix glycoprotein tenascin-C," *Journal of Neuroscience*, vol. 22, no. 16, pp. 7177–7194, 2002.

[198] E. W. Harris and C. W. Cotman, "Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists," *Neuroscience Letters*, vol. 70, no. 1, pp. 132–137, 1986.

[199] M. G. Weisskopf, E. P. Bauer, and J. E. LeDoux, "L-type voltage-gated calcium channels mediate NMDA-independent associative long-term potentiation at thalamic input synapses to the amygdala," *Journal of Neuroscience*, vol. 19, no. 23, pp. 10512–10519, 1999.

[200] A. Kapur, M. F. Yeckel, R. Gray, and D. Johnston, "L-type calcium channels are required for one form of hippocampal mossy fiber LTP," *Journal of Neurophysiology*, vol. 79, no. 4, pp. 2181–2190, 1998.

[201] P. E. Castillo, M. G. Weisskopf, and R. A. Nicoll, "The role of Ca^{2+} channels in hippocampal mossy fiber synaptic transmission and long-term potentiation," *Neuron*, vol. 12, no. 2, pp. 261–269, 1994.

[202] S. Remy and N. Spruston, "Dendritic spikes induce single-burst long-term potentiation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 43, pp. 17192–17197, 2007.

[203] R. E. Westenbroek, L. Hoskins, and W. A. Catterall, "Localization of Ca^{2+} channel subtypes on rat spinal motor neurons, interneurons, and nerve terminals," *Journal of Neuroscience*, vol. 18, no. 16, pp. 6319–6330, 1998.

[204] K. P. Carlin, K. E. Jones, Z. Jiang, L. M. Jordan, and R. M. Brownstone, "Dendritic L-type calcium currents in mouse spinal motoneurons: implications for bistability," *European Journal of Neuroscience*, vol. 12, no. 5, pp. 1635–1646, 2000.

[205] A. Naka, D. Gruber-Schoffnegger, and J. Sandkühler, "Non-Hebbian plasticity at C-fiber synapses in rat spinal cord lamina I neurons," *Pain*, vol. 154, no. 8, pp. 1333–1342, 2013.

[206] M. Tanabe, H. Murakami, M. Honda, and H. Ono, "Gabapentin depresses C-fiber-evoked field potentials in rat spinal dorsal horn only after induction of long-term potentiation," *Experimental Neurology*, vol. 202, no. 2, pp. 280–286, 2006.

[207] K. Hara, Y. Saito, Y. Kirihara, S. Sakura, and Y. Kosaka, "Antinociceptive effects of intrathecal L-type calcium channel blockers on visceral and somatic stimuli in the rat," *Anesthesia and Analgesia*, vol. 87, no. 2, pp. 382–387, 1998.

[208] V. Neugebauer, H. Vanegas, J. Nebe, P. Rümenapp, and H. G. Schaible, "Effects of N- and L-type calcium channel antagonists on the responses of nociceptive spinal cord neurons to mechanical stimulation of the normal and the inflamed knee joint," *Journal of Neurophysiology*, vol. 76, no. 6, pp. 3740–3749, 1996.

[209] A. B. Malmberg and T. L. Yaksh, "Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalin-induced nociception," *Journal of Neuroscience*, vol. 14, no. 8, pp. 4882–4890, 1994.

[210] K. A. Sluka, "Blockade of calcium channels can prevent the onset of secondary hyperalgesia and allodynia induced by intradermal injection of capsaicin in rats," *Pain*, vol. 71, no. 2, pp. 157–164, 1997.

[211] K. A. Sluka, "Blockade of N- and P/Q-type calcium channels reduces the secondary heat hyperalgesia induced by acute inflammation," *Journal of Pharmacology and Experimental Therapeutics*, vol. 287, no. 1, pp. 232–237, 1998.

[212] S. R. Chaplan, J. W. Pogrel, and T. L. Yaksh, "Role of voltage-dependent calcium channel subtypes in experimental tactile allodynia," *Journal of Pharmacology and Experimental Therapeutics*, vol. 269, no. 3, pp. 1117–1123, 1994.

[213] P. Fossat, E. Dobremez, R. Bouali-Benazzouz et al., "Knockdown of L calcium channel subtypes: differential effects in neuropathic pain," *Journal of Neuroscience*, vol. 30, no. 3, pp. 1073–1085, 2010.

[214] A. Favereaux, O. Thoumine, R. Bouali-Benazzouz et al., "Bidirectional integrative regulation of Cav1.2 calcium channel by microRNA miR-103: role in pain," *The EMBO Journal*, vol. 30, no. 18, pp. 3830–3841, 2011.

[215] S. Hatakeyama, M. Wakamori, M. Ino et al., "Differential nociceptive responses in mice lacking the α_{1B} subunit of N-type Ca^{2+} channels," *NeuroReport*, vol. 12, no. 11, pp. 2423–2427, 2001.

[216] D. S. Kim, C. H. Yoon, S. J. Lee, S. Y. Park, H. J. Yoo, and H. J. Cho, "Changes in voltage-gated calcium channel α_1 gene expression in rat dorsal root ganglia following peripheral nerve injury," *Molecular Brain Research*, vol. 96, no. 1-2, pp. 151–156, 2001.

[217] M. Murakami, O. Nakagawasai, T. Suzuki et al., "Antinociceptive effect of different types of calcium channel inhibitors and the distribution of various calcium channel α_1 subunits in the dorsal horn of spinal cord in mice," *Brain Research*, vol. 1024, no. 1-2, pp. 122–129, 2004.

[218] M. Umeda, T. Ohkubo, J. Ono, T. Fukuizumi, and K. Kitamura, "Molecular and immunohistochemical studies in expression of voltage-dependent Ca^{2+} channels in dorsal root ganglia from streptozotocin-induced diabetic mice," *Life Sciences*, vol. 79, no. 21, pp. 1995–2000, 2006.

[219] T. Takahashi and A. Momiyama, "Different types of calcium channels mediate central synaptic transmission," *Nature*, vol. 366, no. 6451, pp. 156–158, 1993.

[220] M. Ogasawara, T. Kurihara, Q. Hu, and T. Tanabe, "Characterization of acute somatosensory pain transmission in P/Q-type Ca^{2+} channel mutant mice, leaner," *FEBS Letters*, vol. 508, no. 2, pp. 181–186, 2001.

[221] K. A. Pelkey, L. Topolnik, J. C. Lacaille, and C. J. McBain, "Compartmentalized Ca^{2+} channel regulation at divergent mossy-fiber release sites underlies target cell-dependent plasticity," *Neuron*, vol. 52, no. 3, pp. 497–510, 2006.

[222] A. Sarihi, J. Mirnajafi-Zadeh, B. Jiang et al., "Cell type-specific, presynaptic LTP of inhibitory synapses on fast-spiking GABAergic neurons in the mouse visual cortex," *Journal of Neuroscience*, vol. 32, no. 38, pp. 13189–13199, 2012.

[223] B. Heinke, E. Balzer, and J. Sandkühler, "Pre- and postsynaptic contributions of voltage-dependent Ca^{2+} channels to nociceptive transmission in rat spinal lamina I neurons," *European Journal of Neuroscience*, vol. 19, no. 1, pp. 103–111, 2004.

[224] T. Yamamoto and Y. Sakashita, "Differential effects of intrathecally administered N- and P-type voltage-sensitive calcium channel blockers upon two models of experimental mononeuropathy in the rat," *Brain Research*, vol. 794, no. 2, pp. 329–332, 1998.

[225] S. Luvisetto, S. Marinelli, M. S. Panasiti et al., "Pain sensitivity in mice lacking the $Ca_v2.1$ α_1 subunit of P/Q-type Ca^{2+} channels," *Neuroscience*, vol. 142, no. 3, pp. 823–832, 2006.

[226] N. Fukumoto, Y. Obama, N. Kitamura et al., "Hypoalgesic behaviors of P/Q-type voltage-gated Ca^{2+} channel mutant mouse, rolling mouse Nagoya," *Neuroscience*, vol. 160, no. 1, pp. 165–173, 2009.

[227] W. Gruner and L. R. Silva, "ω-conotoxin sensitivity and presynaptic inhibition of glutamatergic sensory neurotransmission in vitro," *Journal of Neuroscience*, vol. 14, no. 5, part 1, pp. 2800–2808, 1994.

[228] G. G. T. Holz, K. Dunlap, and R. M. Kream, "Characterization of the electrically evoked release of substance P from dorsal root ganglion neurons: methods and dihydropyridine sensitivity," *Journal of Neuroscience*, vol. 8, no. 2, pp. 463–471, 1988.

[229] A. R. Evans, G. D. Nicol, and M. R. Vasko, "Differential regulation of evoked peptide release by voltage-sensitive calcium channels in rat sensory neurons," *Brain Research*, vol. 712, no. 2, pp. 265–273, 1996.

[230] D. Jeon, C. Kim, Y. M. Yang et al., "Impaired long-term memory and long-term potentiation in N-type Ca^{2+} channel-deficient mice," *Genes, Brain and Behavior*, vol. 6, no. 4, pp. 375–388, 2007.

[231] M. S. Ahmed and S. A. Siegelbaum, "Recruitment of N-type Ca^{2+} channels during LTP enhances low release efficacy of hippocampal CA1 perforant path synapses," *Neuron*, vol. 63, no. 3, pp. 372–385, 2009.

[232] P. Santicioli, E. Del Bianco, M. Tramontana, P. Geppetti, and C. A. Maggi, "Release of calcitonin gene-related peptide like-immunoreactivity induced by electrical field stimulation from

rat spinal afferents is mediated by conotoxin-sensitive calcium channels," *Neuroscience Letters*, vol. 136, no. 2, pp. 161–164, 1992.

[233] C. A. Maggi, M. Tramontana, R. Cecconi, and P. Santicioli, "Neurochemical evidence for the involvement of N-type calcium channels in transmitter secretion from peripheral endings of sensory nerves in guinea pigs," *Neuroscience Letters*, vol. 114, no. 2, pp. 203–206, 1990.

[234] H. Vanegas and H. G. Schaible, "Effects of antagonists to high-threshold calcium channels upon spinal mechanisms of pain, hyperalgesia and allodynia," *Pain*, vol. 85, no. 1-2, pp. 9–18, 2000.

[235] R. J. Winquist, J. Q. Pan, and V. K. Gribkoff, "Use-dependent blockade of $Ca_v2.2$ voltage-gated calcium channels for neuropathic pain," *Biochemical Pharmacology*, vol. 70, no. 4, pp. 489–499, 2005.

[236] H. Saegusa, T. Kurihara, S. Zong et al., "Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel," *The EMBO Journal*, vol. 20, no. 10, pp. 2349–2356, 2001.

[237] T. Fukuizumi, T. Ohkubo, and K. Kitamura, "Spinal sensitization mechanism in vincristine-induced hyperalgesia in mice," *Neuroscience Letters*, vol. 343, no. 2, pp. 89–92, 2003.

[238] E. A. Matthews and A. H. Dickenson, "Effects of spinally delivered N- and P-type voltage-dependent calcium channel antagonists on dorsal horn neuronal responses in a rat model of neuropathy," *Pain*, vol. 92, no. 1-2, pp. 235–246, 2001.

[239] D. Cizkova, J. Marsala, N. Lukacova et al., "Localization of N-type Ca^{2+} channels in the rat spinal cord following chronic constrictive nerve injury," *Experimental Brain Research*, vol. 147, no. 4, pp. 456–463, 2002.

[240] T. J. Bell, C. Thaler, A. J. Castiglioni, T. D. Helton, and D. Lipscombe, "Cell-specific alternative splicing increases calcium channel current density in the pain pathway," *Neuron*, vol. 41, no. 1, pp. 127–138, 2004.

[241] A. Andrade, S. Denome, Y. Q. Jiang, S. Marangoudakis, and D. Lipscombe, "Opioid inhibition of N-type Ca^{2+} channels and spinal analgesia couple to alternative splicing," *Nature Neuroscience*, vol. 13, no. 10, pp. 1249–1256, 2010.

[242] J. M. Brittain, D. B. Duarte, S. M. Wilson et al., "Suppression of inflammatory and neuropathic pain by uncoupling CRMP-2 from the presynaptic Ca^{2+} channel complex," *Nature Medicine*, vol. 17, no. 7, pp. 822–829, 2011.

[243] C. C. Chen, J. W. Shen, N. C. Chung, M. Y. Min, S. J. Cheng, and I. Y. Liu, "Retrieval of context-associated memory is dependent on the $Ca_V3.2$ T-type calcium channel," *PLoS ONE*, vol. 7, no. 1, Article ID e29384, 2012.

[244] Y. Wang, M. J. Rowan, and R. Anwyl, "LTP induction dependent on activation of Ni^{2+}-sensitive voltage-gated calcium channels, but not NMDA receptors, in the rat dentate gyrus in vitro," *Journal of Neurophysiology*, vol. 78, no. 5, pp. 2574–2581, 1997.

[245] Y. Isomura, Y. Fujiwara-Tsukamoto, M. Imanishi, A. Nambu, and M. Takada, "Distance-dependent Ni^{2+}-sensitivity of synaptic plasticity in apical dendrites of hippocampal CA1 pyramidal cells," *Journal of Neurophysiology*, vol. 87, no. 2, pp. 1169–1174, 2002.

[246] D. Song, Z. Wang, and T. W. Berger, "Contribution of T-Type VDCC to TEA-induced long-term synaptic modification in hippocampal CA1 and dentate gyrus," *Hippocampus*, vol. 12, no. 5, pp. 689–697, 2002.

[247] E. Suzuki and T. Okada, "Stratum oriens stimulation-evoked modulation of hippocampal long-term potentiation involves the activation of muscarinic acetylcholine receptors and the inhibition of Kv7/M potassium ion channels," *European Journal of Neuroscience*, vol. 36, no. 1, pp. 1984–1992, 2012.

[248] E. M. Talley, L. L. Cribbs, J. H. Lee, A. Daud, E. Perez-Reyes, and D. A. Bayliss, "Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels," *Journal of Neuroscience*, vol. 19, no. 6, pp. 1895–1911, 1999.

[249] L. Y. M. Huang, "Calcium channels in isolated rat dorsal horn neurones, including labelled spinothalamic and trigeminothalamic cells," *Journal of Physiology*, vol. 411, pp. 161–177, 1989.

[250] P. D. Ryu and M. Randić, "Low- and high-voltage-activated calcium currents in rat spinal dorsal horn neurons," *Journal of Neurophysiology*, vol. 63, no. 2, pp. 273–285, 1990.

[251] M. A. Walsh, B. A. Graham, A. M. Brichta, and R. J. Callister, "Evidence for a critical period in the development of excitability and potassium currents in mouse lumbar superficial dorsal horn neurons," *Journal of Neurophysiology*, vol. 101, no. 4, pp. 1800–1812, 2009.

[252] W. H. Ku and S. P. Schneider, "Multiple T-type Ca^{2+} current subtypes in electrophysiologically characterized hamster dorsal horn neurons: possible role in spinal sensory integration," *Journal of Neurophysiology*, vol. 106, no. 5, pp. 2486–2498, 2011.

[253] E. A. Matthews and A. H. Dickenson, "Effects of ethosuximide, a T-type Ca^{2+} channel blocker, on dorsal horn neuronal responses in rats," *European Journal of Pharmacology*, vol. 415, no. 2-3, pp. 141–149, 2001.

[254] R. Drdla and J. Sandkühler, "Long-term potentiation at C-fibre synapses by low-level presynaptic activity in vivo," *Molecular Pain*, vol. 4, article 18, 2008.

[255] E. Bourinet, A. Alloui, A. Monteil et al., "Silencing of the $Ca_v3.2$ T-type calcium channel gene in sensory neurons demonstrates its major role in nociception," *The EMBO Journal*, vol. 24, no. 2, pp. 315–324, 2005.

[256] R. S. Scroggs and A. P. Fox, "Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size," *Journal of Physiology*, vol. 445, pp. 639–658, 1992.

[257] J.-B. Shin, C. Martinez-Salgado, P. A. Heppenstall, and G. R. Lewin, "A T-type calcium channel required for normal function of a mammalian mechanoreceptor," *Nature Neuroscience*, vol. 6, no. 7, pp. 724–730, 2003.

[258] S. Choi, H. S. Na, J. Kim et al., "Attenuated pain responses in mice lacking $Ca_V3.2$ T-type channels," *Genes, Brain and Behavior*, vol. 6, no. 5, pp. 425–431, 2007.

[259] D. Kim, D. Park, S. Choi et al., "Thalamic control of visceral nociception mediated by T-type Ca^{2+} channels," *Science*, vol. 302, no. 5642, pp. 117–119, 2003.

[260] A. Dogrul, L. R. Gardell, M. H. Ossipov, F. C. Tulunay, J. Lai, and F. Porreca, "Reversal of experimental neuropathic pain by T-type calcium channel blockers," *Pain*, vol. 105, no. 1-2, pp. 159–168, 2003.

[261] X. J. Wen, S. Y. Xu, Z.-X. Chen, C. X. Yang, H. Liang, and H. Li, "The roles of T-type calcium channel in the development of neuropathic pain following chronic compression of rat dorsal root ganglia," *Pharmacology*, vol. 85, no. 5, pp. 295–300, 2010.

[262] S. M. Todorovic, V. Jevtovic-Todorovic, A. Meyenburg et al., "Redox modulation of T-type calcium channels in rat peripheral nociceptors," *Neuron*, vol. 31, no. 1, pp. 75–85, 2001.

[263] S. M. Todorovic, A. Meyenburg, and V. Jevtovic-Todorovic, "Redox modulation of peripheral T-type Ca^{2+} channels in vivo: alteration of nerve injury-induced thermal hyperalgesia," *Pain*, vol. 109, no. 3, pp. 328–339, 2004.

[264] Y. Maeda, Y. Aoki, F. Sekiguchi et al., "Hyperalgesia induced by spinal and peripheral hydrogen sulfide: evidence for involvement of $Ca_v3.2$ T-type calcium channels," *Pain*, vol. 142, no. 1-2, pp. 127–132, 2009.

[265] T. Takahashi, Y. Aoki, K. Okubo et al., "Upregulation of $Ca_v3.2$ T-type calcium channels targeted by endogenous hydrogen sulfide contributes to maintenance of neuropathic pain," *Pain*, vol. 150, no. 1, pp. 183–191, 2010.

[266] M. H. Myoga and W. G. Regehr, "Calcium microdomains near R-type calcium channels control the induction of presynaptic long-term potentiation at parallel fiber to purkinje cell synapses," *Journal of Neuroscience*, vol. 31, no. 14, pp. 5235–5243, 2011.

[267] R. Yasuda, B. L. Sabatini, and K. Svoboda, "Plasticity of calcium channels in dendritic spines," *Nature Neuroscience*, vol. 6, no. 9, pp. 948–955, 2003.

[268] H. Takahashi and J. C. Magee, "Pathway interactions and synaptic plasticity in the dendritic tuft regions of CA1 pyramidal neurons," *Neuron*, vol. 62, no. 1, pp. 102–111, 2009.

[269] J. C. Magee and D. Johnston, "Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons," *Journal of Physiology*, vol. 487, part 1, pp. 67–90, 1995.

[270] Z. Fang, J. H. Hwang, J. S. Kim, S. J. Jung, and S. B. Oh, "R-type calcium channel isoform in rat dorsal root ganglion neurons," *Korean Journal of Physiology and Pharmacology*, vol. 14, no. 1, pp. 45–49, 2010.

[271] E. Bourinet, S. C. Stotz, R. L. Spaetgens et al., "Interaction of SNX482 with domains III and IV inhibits activation gating of α_{1E} ($Ca_V2.3$) calcium channels," *Biophysical Journal*, vol. 81, no. 1, pp. 79–88, 2001.

[272] E. A. Matthews, L. A. Bee, G. J. Stephens, and A. H. Dickenson, "The $Ca_v2.3$ calcium channel antagonist SNX-482 reduces dorsal horn neuronal responses in a rat model of chronic neuropathic pain," *European Journal of Neuroscience*, vol. 25, no. 12, pp. 3561–3569, 2007.

[273] H. Saegusa, T. Kurihara, S. Zong et al., "Altered pain responses in mice lacking α_{1E} subunit of the voltage-dependent Ca^{2+} channel," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 11, pp. 6132–6137, 2000.

[274] R.-R. Ji, T. Kohno, K. A. Moore, and C. J. Woolf, "Central sensitization and LTP: do pain and memory share similar mechanisms?" *Trends in Neurosciences*, vol. 26, no. 12, pp. 696–705, 2003.

[275] K. Deisseroth, "Optogenetics," *Nature Methods*, vol. 8, no. 1, pp. 26–29, 2011.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2013, Article ID 954302, 8 pages
http://dx.doi.org/10.1155/2013/954302

Research Article

Presynaptic Glycine Receptors Increase GABAergic Neurotransmission in Rat Periaqueductal Gray Neurons

Kwi-Hyung Choi, Michiko Nakamura, and Il-Sung Jang

Department of Pharmacology, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea

Correspondence should be addressed to Il-Sung Jang; jis7619@mail.knu.ac.kr

Received 24 May 2013; Revised 6 July 2013; Accepted 31 July 2013

Academic Editor: Dong-ho Youn

The periaqueductal gray (PAG) is involved in the central regulation of nociceptive transmission by affecting the descending inhibitory pathway. In the present study, we have addressed the functional role of presynaptic glycine receptors in spontaneous glutamatergic transmission. Spontaneous EPSCs (sEPSCs) were recorded in mechanically dissociated rat PAG neurons using a conventional whole-cell patch recording technique under voltage-clamp conditions. The application of glycine (100 μM) significantly increased the frequency of sEPSCs, without affecting the amplitude of sEPSCs. The glycine-induced increase in sEPSC frequency was blocked by 1 μM strychnine, a specific glycine receptor antagonist. The results suggest that glycine acts on presynaptic glycine receptors to increase the probability of glutamate release from excitatory nerve terminals. The glycine-induced increase in sEPSC frequency completely disappeared either in the presence of tetrodotoxin or Cd^{2+}, voltage-gated Na^{+}, or Ca^{2+} channel blockers, suggesting that the activation of presynaptic glycine receptors might depolarize excitatory nerve terminals. The present results suggest that presynaptic glycine receptors can regulate the excitability of PAG neurons by enhancing glutamatergic transmission and therefore play an important role in the regulation of various physiological functions mediated by the PAG.

1. Introduction

Glycine, in addition to GABA, is the primary inhibitory neurotransmitter in the brain stem and spinal cord. In mature neurons, the inhibitory action of glycine is accomplished by activating strychnine-sensitive glycine receptors and opening Cl^{-} channels, which results in membrane shunting or hyperpolarization of postsynaptic neurons [1]. Glycine receptors are found in most of brain areas including the hippocampus, amygdala, ventral tegmental area, and periaqueductal gray (PAG) [2–5]. Nevertheless, functional roles of glycine receptors are largely unknown because glycine is unlikely to be released from presynaptic nerve terminals, and there is no direct evidence for glycinergic inhibitory postsynaptic currents in these brain structures (but see also [6]). However, previous studies have shown that endogenous glycine and/or taurine can elicit the tonic Cl^{-} currents mediated by glycine receptors in central neurons [5, 7], suggesting that endogenous glycine and/or taurine may play a role in the regulation of neuronal excitability. On the other hand, glycine receptors are also found in presynaptic nerve terminals of many brain regions, and their activation is known to facilitate neurotransmitter release from presynaptic nerve terminals [8–11]. In these cases, presynaptic glycine receptors might regulate the neuronal excitability in an indirect manner via the presynaptic modulation of neurotransmitter release.

The PAG is involved in the various functions including pain, vocalization, fear and anxiety, lordosis, and cardiovascular control [12, 13]. In particular, the PAG plays a crucial role in the regulation of nociceptive transmission as the PAG is one of regulatory centers affecting the endogenous descending inhibitory pathway such as noradrenergic and serotonergic systems [13]. In fact, electrical stimulation of the PAG region reduces neuropathic pain by activating the descending inhibitory system [14, 15]. In addition, the PAG is known to be one of the target sites for opioids and cannabinoids [16, 17]. On the other hand, it has been well established that several neurotransmitters including glutamate and

GABA within the PAG are responsible for the regulation of nociceptive transmission [13, 18]. Of them, glycine is likely to play a role in the processing of pain within the PAG, as glycine is inversely correlated to nociceptive paw stimulation [19]. However, a significant amount of the glycine released within the PAG seems to act on glycine sites of NMDA receptors rather than strychnine-sensitive glycine receptors [20–22], indicating that the functional roles of glycine receptors in the PAG are still largely unknown. In the present study, therefore, we have investigated whether functional glycine receptors exist on glutamatergic nerve terminals projecting to PAG neurons and whether their activation modulates spontaneous glutamatergic transmission.

2. Materials and Methods

2.1. Preparation. All experiments complied with the guiding principles for the care and use of animals approved by the Council of the Physiological Society of Korea and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and every effort was made to minimize both the number of animals used and their suffering.

Sprague Dawley rats (12–16 d old, either sex) were decapitated under ketamine anesthesia (100 mg/kg, i.p.). The midbrain was dissected and transversely sliced at a thickness of 400 μm using a microslicer (VT1000S; Leica, Nussloch, Germany). The midbrain slices containing the PAG were kept in an incubation solution (in mM: 124NaCl, 3KCl, 1.5KH_2PO_4, 24$NaHCO_3$, 2$CaCl_2$, 1.3$MgSO_4$, and 10 glucose) saturated with 95% O_2 and 5% CO_2 at room temperature (22–24°C) for at least 1 h before the mechanical dissociation. For dissociation, slices were transferred into a 35 mm culture dish (Primaria 3801; Becton Dickinson, Rutherford, NJ, USA) containing a standard external solution (in mM: 150NaCl, 3KCl, 2$CaCl_2$, 1$MgCl_2$, 10 glucose, 10 Hepes, and pH 7.4 with Tris-base), and the PAG region was identified under a binocular microscope (SMZ-1; Nikon, Tokyo, Japan). Details of the mechanical dissociation have been described previously [23, 24]. Briefly, mechanical dissociation was accomplished using a custom-built vibration device and a fire-polished glass pipette oscillating at about 50–60 Hz (0.3–0.5 mm) on the surface of the ventrolateral PAG region. Slices were removed and the mechanically dissociated neurons were left for 15 min to allow the neurons to adhere to the bottom of the culture dish.

2.2. Electrophysiology. All electrophysiological measurements were performed using conventional whole-cell patch recording mode at holding potentials (V_H values) of −60 to −65 mV, which are the reversal potential of glycine-induced membrane currents determined in every PAG neurons, except where indicated (Axopatch 200B; Molecular Devices, Union City, CA, USA). Patch pipettes were made from borosilicate capillary glass (1.5 mm outer diameter, 0.9 mm inner diameter; G-1.5; Narishige, Tokyo, Japan) by use of a pipette puller (P-97; Sutter Instrument Co., Novato, CA, USA). The tip of pipette was firstly filled with the Cs-methanesulfonate-based internal solution (in mM: 140 Cs-methanesulfonate, 10CsCl, 2 EGTA, 5 QX-314, 2 ATP-Mg, 10 Hepes, and pH 7.2 with Tris-base) using a capillary phenomenon, and then the CsF-based internal solution, in which Cs-methanesulfonate was replaced with equimolar CsF, was backfilled using a syringe. The resistance of the recording pipettes filled with these internal solutions was 4–6 MΩ. The liquid junction potential (~−11 mV, measured by exchanging bath solution from internal solution to standard external solution) and pipette capacitance were compensated for. Neurons were viewed under phase contrast on an inverted microscope (TE2000; Nikon). Membrane currents were filtered at 2 kHz, digitized at 5 kHz, and stored on a computer equipped with pCLAMP 10.2 (Molecular Devices). During the recordings, 10 mV hyperpolarizing step pulses (30 ms in duration) were periodically applied to monitor the access resistance. All experiments were performed at room temperature (22–25°C).

2.3. Data Analysis. Spontaneous excitatory postsynaptic currents (sEPSCs) were counted and analyzed using the Mini-Analysis program (Synaptosoft, Inc., Decatur, GA, USA) as described previously [25]. Briefly, sEPSCs were screened automatically using an amplitude threshold of 10 pA and then were visually accepted or rejected based upon the rise and decay times. Basal noise levels during voltage-clamp recordings were typically less than 8 pA. The average values of the frequency, amplitude, and decay time constant (90–37%) of sEPSCs during the control period or each drug condition (5 min) were calculated for each recording, and the frequency and amplitude of all the events during the glycine application (1-2 min) were normalized to these values. The effects of these different conditions were quantified as a percentage increase in sEPSC frequency compared to the control values. The interevent intervals and amplitudes of a large number of synaptic events obtained from the same neuron were examined by constructing cumulative probability distributions and compared using the Kolmogorov-Smirnov (K-S) test with Stat View software (SAS Institute, Inc., Cary, NC, USA). Numerical values are expressed as the mean ± standard error of the mean (SEM) using values normalized to the control. Significant differences in the mean amplitude and frequency were tested using Student's paired two-tailed t-test, using absolute values rather than normalized ones. Values of $P < 0.05$ were considered significant.

2.4. Drugs. The drugs used in the present study were glycine, strychnine, 6-imino-3-(4-methoxyphenyl)-1(6H)-pyridazinebutanoic acid HBr (SR95531), tetrodotoxin (TTX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-2-amino-5-phosphonovaleric acid (APV), QX-314, EGTA, $CdCl_2$, and ATP-Mg (from Sigma, St. Louis, MO, USA). The standard external solution routinely contained 10 μM SR95531 and APV 50 μM APV to block $GABA_A$ and NMDA receptors, respectively. All solutions containing drugs were applied using the "Y-tube system" for rapid solution exchange [26].

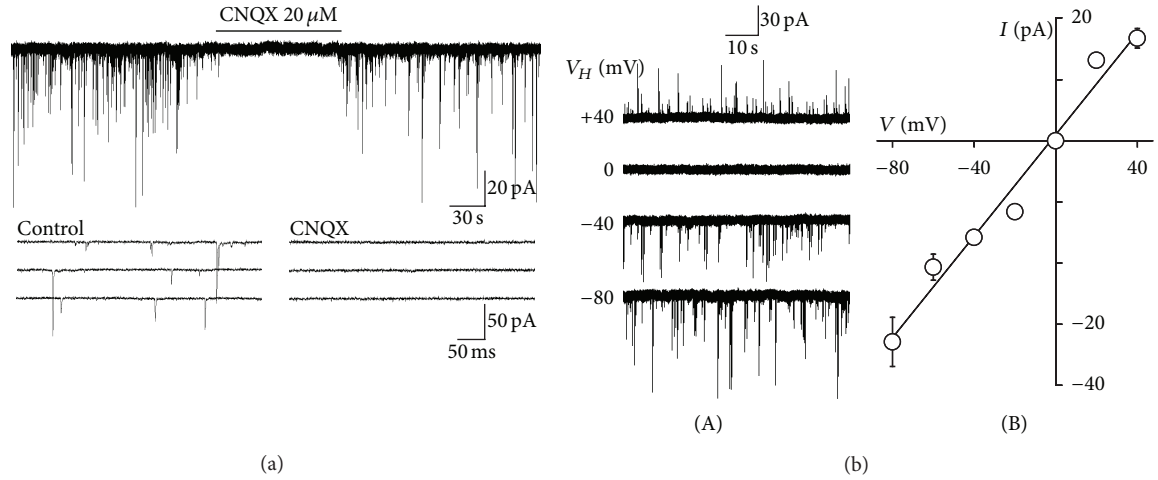


Figure 1: Glutamatergic sEPSCs recorded from acutely isolated PAG neurons. (a) A typical trace of glutamatergic sEPSCs observed before, during, and after application of 20 μM CNQX, an AMPA/KA receptor blocker, at a V_H of 0 mV in the presence of 10 μM SR95531 and 50 μM APV, selective $GABA_A$, and NMDA receptor antagonists, respectively. Insets represent typical traces with an expanded time scale. (b) (A) Typical traces of glutamatergic sEPSCs at various holding potentials (V_H). (B) A plot of the mean amplitude of sEPSCs at various V_H values. The reversal potential was −2.5 mV, which is close to the theoretical equilibrium potential of monovalent cations. Each point was the mean and SEM from the 4 neurons.

3. Results

After brief mechanical dissociation of the ventrolateral PAG region, several kinds of neurons that differed in soma diameter (10–15 μm) and shape (multipolar, bipolar, and pyramidal-shaped) were found. These morphological properties of acutely isolated neurons were similar to those of PAG neurons identified in previous studies [27, 28]. When these neurons were held at a V_H of −60 mV using the whole-cell patch-clamp technique, the spontaneous inward synaptic currents were recorded in the presence of both 10 μM SR95531 and 50 μM APV, selective $GABA_A$ and NMDA, and receptor antagonists, respectively. These spontaneous inward currents were completely and reversibly blocked by 20 μM CNQX ($n = 5$), an AMPA/KA receptor blocker (Figure 1(a)). Figure 1(b) shows typical raw traces recorded at various V_H conditions and the current-voltage relationship ($n = 4$). The reversal potential for the spontaneous synaptic currents was estimated from the current-voltage relationship to be −2.5 mV. This value is very similar to the theoretical equilibrium potential of monovalent cations. These results indicate that the spontaneous synaptic events recorded from acutely isolated PAG neurons were AMPA/KA receptor-mediated sEPSCs.

To investigate whether excitatory nerve terminals projecting to PAG neurons express functional glycine receptors and whether the activation of presynaptic glycine receptors directly modulates spontaneous glutamate release, we observed the effect of exogenously applied glycine on sEPSCs. The glycine receptor-mediated membrane currents were minimized by using the CsF-based pipette solution and by adjusting the V_H to experimentally determined reversal potential of glycine-induced currents. In these conditions, glycine (100 μM) rapidly and reversibly increased the frequency of glutamatergic sEPSCs (Figure 2(a)). In 12 neurons for which the effect was fully analyzed, glycine (100 μM) increased sEPSC frequency to 429.7 ± 33.9% of the control (0.81 ± 0.18 Hz for control and 3.48 ± 0.27 Hz for glycine, $P < 0.01$), without affecting sEPSC amplitude (98.3 ± 5.9% of the control, 23.1 ± 1.9 pA for control, and 22.7 ± 1.6 pA for glycine, $P = 0.57$; Figures 2(a) and 2(b) insets). In addition, glycine significantly shifted the cumulative distribution of interevent interval to the left ($P < 0.01$, K-S test, Figure 2(b)(A)) without affecting the cumulative distribution of the current amplitude ($P = 0.13$, K-S test, Figure 2(b)(B)), consistent with an increase in the frequency of glutamatergic sEPSCs. Glycine also did not affect the decay time constant of glutamatergic sEPSCs (2.21 ± 0.12 ms of the control and 2.19 ± 0.13 ms for glycine, $P = 0.96$; Figure 2(a) inset). Taken together, these results suggest that glycine acts presynaptically to increase spontaneous glutamate release onto acutely isolated PAG neurons.

To investigate whether the glycine-induced increase in spontaneous glutamate release is mediated by presynaptic glycine receptors, we observed the effect of strychnine, a specific glycine receptor antagonist, on the glycine-induced increase in sEPSC frequency. Strychnine (1 μM) by itself had no effect on the basal frequency (111.6 ± 10.3% of the control, $n = 6$, $P = 0.31$) or amplitude (97.6 ± 8.5% of the control, $n = 6$, $P = 0.21$) of glutamatergic sEPSCs (Figures 3(a) and 3(b)). In the presence of 1 μM strychnine, the facilitatory action of glycine (418.1 ± 38.3% of the control, $n = 6$, $P < 0.01$) was completely attenuated to 88.4 ± 9.2% of the strychnine condition ($n = 6$, $P = 0.42$, Figures 3(a) and 3(b)(A)).

Next, the possible mechanisms underlying the glycine-induced increase in spontaneous glutamate release were examined. Since the activation of presynaptic glycine receptors facilitates spontaneous neurotransmitter release by eliciting a presynaptic depolarization [9–11], we observed

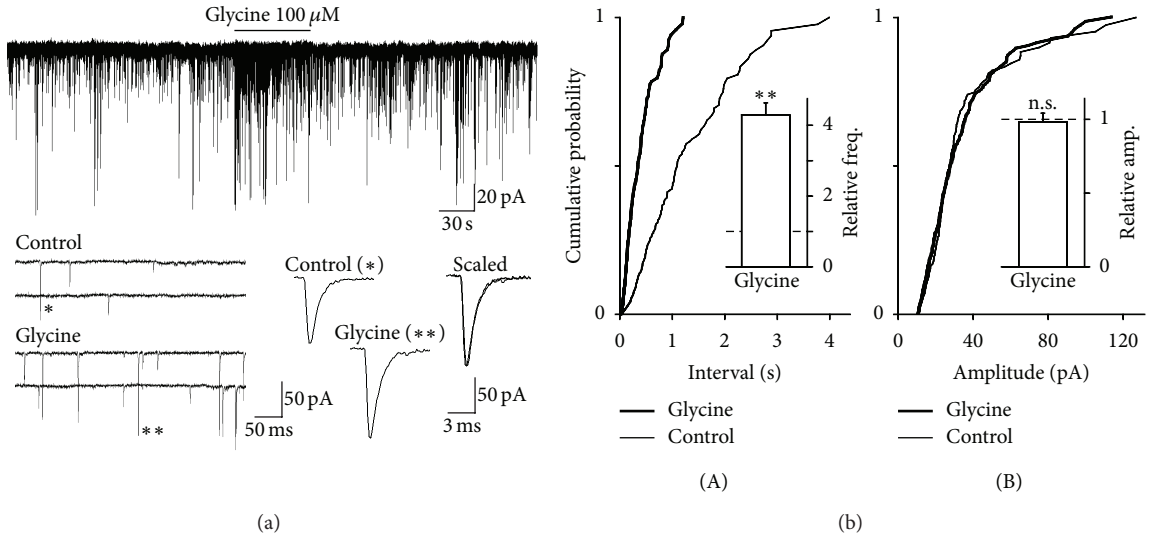


Figure 2: Effects of glycine on glutamatergic sEPSCs. (a) A typical trace of glutamatergic sEPSCs observed before, during, and after application of 100 μM glycine. Insets represent typical traces with an expanded time scale (left) and single sEPSCs indicated by symbols (right). (b) Cumulative probability distribution for interevent interval (A) and current amplitude (B) of glutamatergic sEPSCs. 191 for control (thin lines) and 292 events for glycine (thick lines) were plotted. Insets column represents mean and SEM from 12 neurons. Dotted lines represent the relative control of basal frequency and amplitude of sEPSCs. $^{**}P < 0.01$; n.s.: not significant.

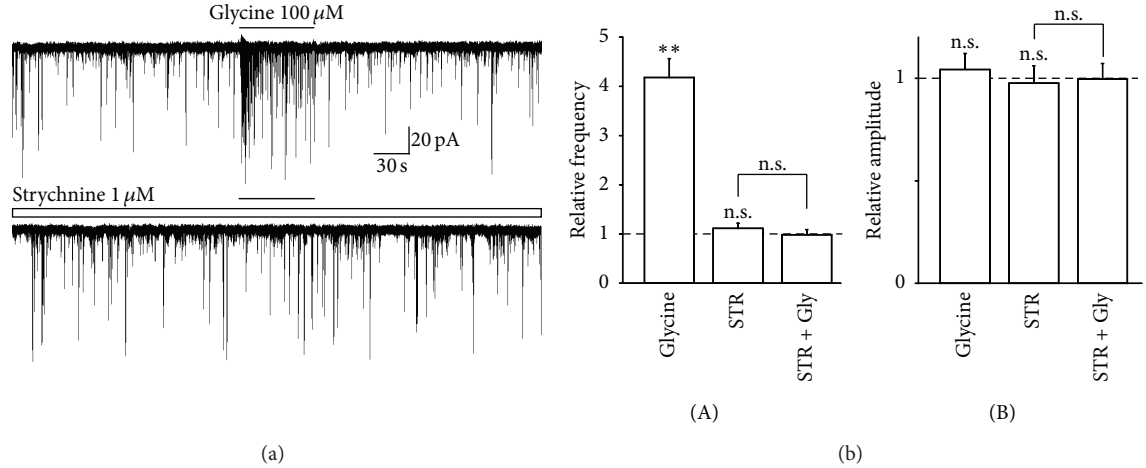


Figure 3: Effect of strychnine on glycine-induced increase in sEPSC frequency. (a) Typical traces of glutamatergic sEPSCs observed during the application of 100 μM glycine in the absence (upper) and presence (lower) of 1 μM strychnine. (b) Glycine-induced changes in frequency (A) and amplitude (B) of sEPSC in the absence and presence of strychnine. Each column was the mean and SEM from 6 neurons. $^{**}P < 0.01$; n.s.: not significant.

the effect of TTX, a voltage-dependent Na^+ channel blocker, on the glycine-induced increase in sEPSCs frequency. The application of 300 nM TTX significantly decreased the basal sEPSC frequency (64.4 ± 4.6% of the control, $n = 6$, $P < 0.01$, Figures 4(a) and 4(b)(A)), but it had no effect on the basal sEPSC amplitude (98.9 ± 8.1% of the control, $n = 6$, $P = 0.17$, Figures 4(a) and 4(b)(B)). In the presence of 300 nM TTX, the facilitatory action of glycine (458.6 ± 39.1% of the control, $n = 6$, $P < 0.01$) was completely occluded to 103.7 ± 11.0% of the TTX condition ($n = 6$, $P = 0.55$, Figures 4(a) and 4(b)(A)).

The neurotransmitter release is triggered by an increase in the intraterminal Ca^{2+} concentration, which is generally accomplished by presynaptic voltage-dependent Ca^{2+} channels (VDCCs) [29]. Therefore, we further examined the effect of Cd^{2+}, a general VDCC blocker, on the glycine-induced increase in sEPSCs frequency. The application of 200 μM Cd^{2+} also significantly decreased the basal sEPSC frequency (61.1 ± 5.5% of the control, $n = 6$, $P < 0.01$, Figures 4(a) and 4(b)(A)). However, Cd^{2+} did not affect the basal sEPSC amplitude (96.2 ± 8.8% of the control, $n = 6$, $P = 0.61$, Figures 5(a) and 5(b)(B)). In the presence of 200 μM Cd^{2+},

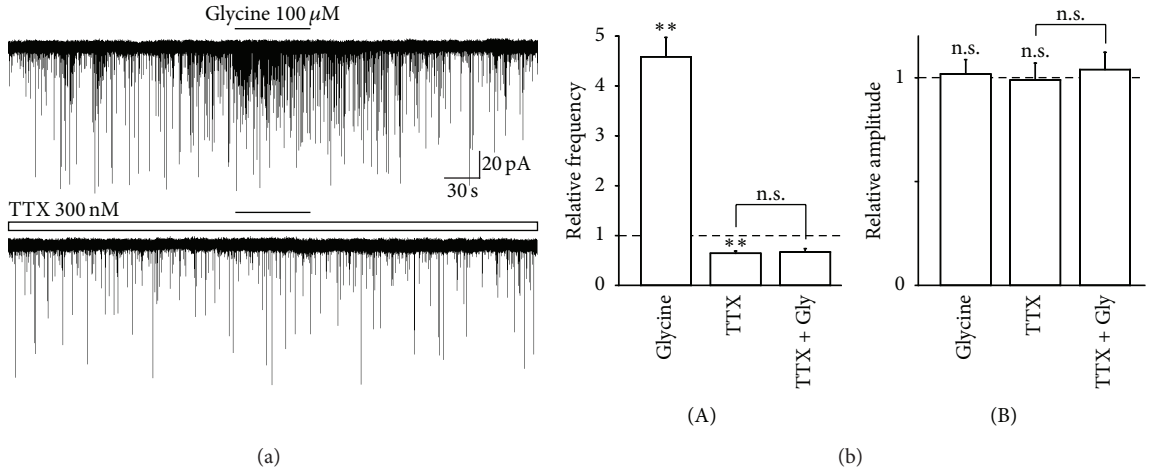


Figure 4: Effect of TTX on glycine-induced increase in sEPSC frequency. (a) Typical traces of glutamatergic sEPSCs observed during the application of 100 μM glycine in the absence (upper) and presence (lower) of 300 nM TTX. (b) Glycine-induced changes in frequency (A) and amplitude (B) of sEPSC in the absence and presence of TTX. Note that the glycine-induced facilitation of sEPSC frequency was completely suppressed by TTX. Each column was the mean and SEM from 7 neurons. $^{**}P < 0.01$; n.s.: not significant.

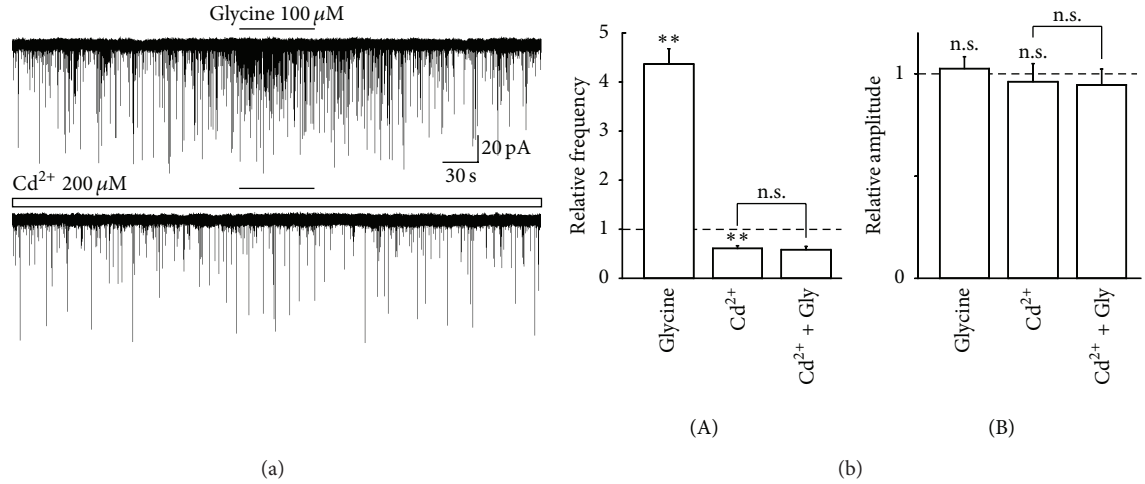


Figure 5: Effect of Cd^{2+} on glycine-induced increase in sEPSC frequency. (a) Typical traces of glutamatergic sEPSCs observed during the application of 100 μM glycine in the absence (upper) and presence (lower) of 200 μM Cd^{2+}. (b) Glycine-induced changes in frequency (A) and amplitude (B) of sEPSC in the absence and presence of Cd^{2+}. Note that the glycine-induced facilitation of sEPSC frequency was completely suppressed by Cd^{2+}. Each column was the mean and SEM from 7 neurons. $^{**}P < 0.01$; n.s.: not significant.

the facilitatory action of glycine (436.8 ± 31.1% of the control, $n = 6$, $P < 0.01$) was completely occluded to 95.9 ± 10.6% of the Cd^{2+} condition ($n = 6$, $P = 0.28$, Figures 5(a) and 5(b)(B)).

4. Discussion

Previous studies have shown that glycine receptors are expressed on presynaptic nerve terminals at central synapses and that their activation modulates the presynaptic release of a variety of neurotransmitters, such as glutamate [8, 11], GABA [10], and glycine [9]. Several lines of evidence suggest that glycine receptors are also expressed on excitatory nerve terminals projecting to PAG neurons and that their activation enhances spontaneous glutamate release onto PAG neurons. First, glycine significantly increased the frequency of sEPSCs without affecting the current amplitude, consistent with a presynaptic locus of glycine action. Second, this facilitatory action of glycine on glutamatergic sEPSCs was completely blocked by strychnine. Although presynaptic $GABA_A$ receptors also enhance spontaneous glutamate release at these synapses [30], the involvement of presynaptic $GABA_A$ receptors should be negligible because the present study was performed after the blockade of $GABA_A$ receptors with SR95531. In addition, since the extracellular solution contained APV, a specific NMDA receptor antagonist, the involvement of possible NMDA receptors should be also negligible. Third, the preparation used in this study would

support a presynaptic locus of glycine action because dissociated neurons have presynaptic nerve terminals without their parent soma [24].

In the present study, we found that glycine failed to enhance sEPSC frequency in the presence of either TTX or Cd^{2+}, suggesting that the glycine-induced increase in sEPSC frequency requires the activation of voltage-dependent Na^+ and Ca^{2+}channels. That is, the activation of presynaptic glycine receptors might depolarize excitatory nerve terminals, and that this presynaptic depolarization seems to activate voltage-dependent Na^+ and Ca^{2+} channels subsequently. In addition, since glycine had no facilitatory effect on spontaneous glutamate release in the presence of TTX, the extent of glycine receptor-mediated presynaptic depolarization might be not enough to activate VDCCs directly [25, 31]. Alternatively, glycine receptors might be expressed on preterminal region so that the glycine receptor-mediated depolarization would affect voltage-dependent Na^+ rather than Ca^{2+} channels at axons. Similarly, nicotinic acetylcholine receptors expressed on the axonal region are known to enhance spontaneous neurotransmitter release in a TTX-sensitive manner [32]. On the other hand, given that glycine receptors are permeable to Cl^- but not cations and that the activation of glycine receptors elicits a presynaptic depolarization; excitatory nerve terminals projecting to PAG neurons might maintain higher intraterminal Cl^- concentration than that predicted for passive Cl^- distribution. This can be accomplished by the inwardly directed Cl^- cotransporters such as bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporter type 1 [33, 34]. Similarly, we have previously shown that bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporter type 1 maintains the higher Cl^- concentration within presynaptic nerve terminals [25, 35]. In this regard, since the Cl^- concentration within the neuronal soma becomes lower with postnatal development by changing the expression of Cl^- cotransporters [36], it is of interest to examine whether the expression of presynaptic Cl^- cotransporters as well as the glycine receptor-mediated presynaptic modulation alters during postnatal development.

As PAG neurons project their excitatory axon terminals directly to serotonergic and noradrenergic neurons of the medulla, which innervate their fibers the superficial dorsal horn [37], the excitability of PAG neurons should be a key factor involved in the PAG-mediated descending inhibitory systems. For example, microinjection of the $GABA_A$ receptor antagonists or glutamate into the PAG shows antinociceptive responses in animal models [38–40]. In addition, opioid analgesics seem to disinhibit tonically active GABAergic neurons within the PAG [41], suggesting that an increase in the excitability of output PAG neurons produces analgesia. In this regard, glycine might be also involved in the regulation of excitability of PAG neurons. For example, a previous study has shown that the microinjection of glycine into the dorsal PAG of rats increases tail-flick latencies in a dose-dependent manner, and this hyponociceptive effect of glycine is reversed by coadministration with the specific inhibitor for NMDA receptor glycine site [42], suggesting that microinjected glycine acts on glycine-binding site of NMDA receptors to elicit hyponociception. In addition, a recent study has shown that the microinjection of glycine into the ventrolateral PAG of rats produces conflicting results, for example, hyperalgesia or analgesia [43]. In this study, while the glycine-induced analgesia is blocked by the NMDA receptor antagonist, the glycine-induced hyperalgesia is blocked by the glycine receptor antagonist [43], suggesting that glycine acts as an excitatory transmitter, for example, coagonist for NMDA receptors, to increase the excitability of output PAG neurons. In the case of glycine-induced hyperalgesia, the activation of glycine receptors, presumably somatodendritic and/or postsynaptic glycine receptors, might result from the decrease in the excitability of output PAG neurons. Although the source of extracellular glycine remains to be elucidated, glycine might be synaptically released as described previously [44]. It should be noted that, however, postsynaptic glycine receptors are unlikely to contribute to the regulation of neuronal excitability, as inhibitory postsynaptic currents are absolutely mediated by $GABA_A$ receptors rather than strychnine-sensitive glycine receptors [45].

In the present study, we have shown that the activation of presynaptic glycine receptors increases spontaneous glutamate release onto PAG neurons via a presynaptic depolarization. The present results would provide a physiological role of presynaptic glycine receptors in the antinociceptive function mediated by the PAG, as the activation of presynaptic glycine receptors can increase the excitability of PAG neurons by enhancing excitatory glutamatergic transmission. This speculation might be different from previous findings showing that glycine microinjected into the PAG produces hyperalgesic action in a strychnine-sensitive manner [43]. However, the previous behavioral findings might be not applicable to the present study because the microinjected glycine can activate somatodendritic as well as presynaptic glycine receptors within the PAG region. Although it is still unknown whether the glycine-induced hyperalgesia is mediated by somatodendritic or presynaptic glycine receptors, somatodendritic glycine receptors might be responsible for the microinjected glycine-induced hyperalgesia. This is because the glycine-induced hyperpolarization decreases the excitability of output PAG neurons, as described above. In fact, PAG neurons express functional somatodendritic glycine receptors, and the application of glycine to isolated PAG neurons elicits large Cl^- currents [46]. Further electrophysiological and behavioral studies will be needed to elucidate the differential roles of somatodendritic and presynaptic glycine receptors in the regulation of nociceptive transmission mediated by the PAG.

5. Conclusions

In conclusion, we have shown that functional glycine receptors are expressed on glutamatergic nerve terminals projecting to PAG neurons and that the activation of presynaptic glycine receptors depolarizes presynaptic terminals to enhance spontaneous glutamate release. The present results suggest that presynaptic glycine receptors can regulate the excitability of PAG neurons by enhancing glutamatergic transmission and therefore play an important role in the

regulation various physiological functions mediated by the PAG.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A2A2A02046812 and 2012-0009327). K.-H. Choi and M. Nakamura have equally contributed to this work.

References

[1] J. W. Lynch, "Molecular structure and function of the glycine receptor chloride channel," *Physiological Reviews*, vol. 84, no. 4, pp. 1051–1095, 2004.

[2] B. A. McCool and J. S. Farroni, "Subunit composition of strychnine-sensitive glycine receptors expressed by adult rat basolateral amygdala neurons," *European Journal of Neuroscience*, vol. 14, no. 7, pp. 1082–1090, 2001.

[3] F. Wang, C. Xiao, and J. H. Ye, "Taurine activates excitatory non-synaptic glycine receptors on dopamine neurones in ventral tegmental area of young rats," *Journal of Physiology*, vol. 565, no. 2, pp. 503–516, 2005.

[4] B. I. Min, C. J. Kim, J. S. Rhee, and N. Akaike, "Modulation of glycine-induced chloride current in acutely dissociated rat periaqueductal gray neurons by μ-opioid agonist, DAGO," *Brain Research*, vol. 734, no. 1-2, pp. 72–78, 1996.

[5] L. H. Zhang, N. Gong, D. Fei, L. Xu, and T. L. Xu, "Glycine uptake regulates hippocampal network activity via glycine receptor-mediated tonic inhibition," *Neuropsychopharmacology*, vol. 33, no. 3, pp. 701–711, 2008.

[6] A. A. Ghavanini, D. A. Mathers, and E. Puil, "Glycinergic inhibition in thalamus revealed by synaptic receptor blockade," *Neuropharmacology*, vol. 49, no. 3, pp. 338–349, 2005.

[7] M. Mori, B. H. Gähwiler, and U. Gerber, "β-Alanine and taurine as endogenous agonists at glycine receptors in rat hippocampus in vitro," *Journal of Physiology*, vol. 539, no. 1, pp. 191–200, 2002.

[8] R. Turecek and L. O. Trussell, "Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse," *Nature*, vol. 411, no. 6837, pp. 587–590, 2001.

[9] H. J. Jeong, I. S. Jang, A. J. Moorhouse, and N. Akaike, "Activation of presynaptic glycine receptors facilitates glycine release from presynaptic terminals synapsing onto rat spinal sacral dorsal commissural nucleus neurons," *Journal of Physiology*, vol. 550, no. 2, pp. 373–383, 2003.

[10] J. H. Ye, F. Wang, K. Krnjević, W. Wang, Z. G. Xiong, and J. Zhang, "Presynaptic glycine receptors on GABAergic terminals facilitate discharge of dopaminergic neurons in ventral tegmental area," *Journal of Neuroscience*, vol. 24, no. 41, pp. 8961–8974, 2004.

[11] E. A. Lee, J. H. Cho, I. S. Choi et al., "Presynaptic glycine receptors facilitate spontaneous glutamate release onto hilar neurons in the rat hippocampus," *Journal of Neurochemistry*, vol. 109, no. 1, pp. 275–286, 2009.

[12] M. M. Behbehani, "Functional characterstics of the midbrain periaqueductal gray," *Progress in Neurobiology*, vol. 46, no. 6, pp. 575–605, 1995.

[13] M. J. Millan, "Descending control of pain," *Progress in Neurobiology*, vol. 66, no. 6, pp. 355–474, 2002.

[14] D. V. Reynolds, "Surgery in the rat during electrical analgesia induced by focal brain stimulation," *Science*, vol. 164, no. 3878, pp. 444–445, 1969.

[15] R. Monhemius, D. L. Green, M. H. T. Roberts, and J. Azami, "Periaqueductal grey mediated inhibition of responses to noxious stimulation is dynamically activated in a rat model of neuropathic pain," *Neuroscience Letters*, vol. 298, no. 1, pp. 70–74, 2001.

[16] T. L. Yaksh, "Pharmacology and mechanisms of opioid analgesic activity," *Acta Anaesthesiologica Scandinavica*, vol. 41, no. 1, pp. 94–111, 1997.

[17] D. P. Finn, M. D. Jhaveri, S. R. G. Beckett et al., "Effects of direct periaqueductal grey administration of a cannabinoid receptor agonist on nociceptive and aversive responses in rats," *Neuropharmacology*, vol. 45, no. 5, pp. 594–604, 2003.

[18] M. M. Behbehani and H. L. Fields, "Evidence that an excitatory connection between the periaqueductal gray and nucleus raphe magnus mediates stimulation produced analgesia," *Brain Research*, vol. 170, no. 1, pp. 85–93, 1979.

[19] S. Maione, I. Marabese, F. S. Rossi et al., "Effects of persistent nociception on periaqueductal gray glycine release," *Neuroscience*, vol. 97, no. 2, pp. 311–316, 2000.

[20] L. Berrino, S. Vitagliano, S. Maione, E. Lampa, and F. Rossi, "Modulation by glycine on vascular effects of NMDA: in vivo experimental research," *Amino Acids*, vol. 5, no. 2, pp. 239–244, 1993.

[21] E. Viu, A. Zapata, J. L. Capdevila, L. H. Fossom, P. Skolnick, and R. Trullas, "Glycine site antagonists and partial agonists inhibit N-methyl-D-aspartate receptor-mediated [3H]arachidonic acid release in cerebellar granule cells," *Journal of Pharmacology and Experimental Therapeutics*, vol. 285, no. 2, pp. 527–532, 1998.

[22] M. A. Martins, A. P. Carobrez, and C. R. Tonussi, "Activation of dorsal periaqueductal gray by glycine produces long lasting hyponociception in rats without overt defensive behaviors," *Life Sciences*, vol. 83, no. 3-4, pp. 118–121, 2008.

[23] J. S. Rhee, H. Ishibashi, and N. Akaike, "Calcium channels in the GABAergic presynaptic nerve terminals projecting to Meynert neurons of the rat," *Journal of Neurochemistry*, vol. 72, no. 2, pp. 800–807, 1999.

[24] N. Akaike and A. J. Moorhouse, "Techniques: applications of the nerve-bouton preparation in neuropharmacology," *Trends in Pharmacological Sciences*, vol. 24, no. 1, pp. 44–47, 2003.

[25] I. S. Jang, H. J. Jeong, S. Katsurabayashi, and N. Akaike, "Functional roles of presynaptic GABAA receptors on glycinergic nerve terminals in the rat spinal cord," *Journal of Physiology*, vol. 541, no. 2, pp. 423–434, 2002.

[26] K. Murase, P. D. Ryu, and M. Randic, "Excitatory and inhibitory amino acids and peptide-induced responses in acutely isolated rat spinal dorsal horn neurons," *Neuroscience Letters*, vol. 103, no. 1, pp. 56–63, 1989.

[27] P. W. Mantyh, "The midbrain periaqueductal gray in the rat, cat, and monkey: a Nissl, Weil, and Golgi analysis," *Journal of Comparative Neurology*, vol. 204, no. 4, pp. 349–363, 1982.

[28] A. J. Beitz and R. D. Shepard, "The midbrain periaqueductal gray in the rat. II. A Golgi analysis," *Journal of Comparative Neurology*, vol. 237, no. 4, pp. 460–475, 1985.

[29] L. G. Wu and P. Saggau, "Presynaptic inhibition of elicited neurotransmitter release," *Trends in Neurosciences*, vol. 20, no. 5, pp. 204–212, 1997.

[30] I. S. Jang, "GABAA receptors facilitate spontaneous glutamate release in rat periaqueductal gray neurons," *NeuroReport*, vol. 22, no. 16, pp. 834–838, 2011.

[31] M. M. Dorostkar and S. Boehm, "Presynaptic lonotropic receptors," *Handbook of Experimental Pharmacology*, vol. 184, pp. 479–527, 2008.

[32] M. Alkondon, E. F. R. Pereira, H. M. Eisenberg, and E. X. Albuquerque, "Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that modulate GABA release from CA1 interneurons in rat hippocampal slices," *Journal of Neuroscience*, vol. 19, no. 7, pp. 2693–2705, 1999.

[33] G. H. Clayton, G. C. Owens, J. S. Wolff, and R. L. Smith, "Ontogeny of cation-Cl^- cotransporter expression in rat neocortex," *Developmental Brain Research*, vol. 109, no. 2, pp. 281–292, 1998.

[34] M. D. Plotkin, E. Y. Snyder, S. C. Hebert, and E. Delpire, "Expression of the Na-K-2Cl cotransporter is developmentally regulated in postnatal rat brain: a possible mechanism underlying GABA's excitatory role in immature brain," *Journal of Neurobiology*, vol. 33, no. 6, pp. 781–795, 1997.

[35] I. S. Jang, H. J. Jeong, and N. Akaike, "Contribution of the Na-K-Cl cotransporter on GABAA receptor-mediated presynaptic depolarization in excitatory nerve terminals," *Journal of Neuroscience*, vol. 21, no. 16, pp. 5962–5972, 2001.

[36] C. Rivera, J. Voipio, J. A. Payne et al., "The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation," *Nature*, vol. 397, no. 6716, pp. 251–255, 1999.

[37] A. A. Cameron, I. A. Khan, K. N. Westlund, and W. D. Willis, "The efferent projections of the periaqueductal gray in the rat: a Phaseolus vulgaris-leucoagglutinin study. II. Descending projections," *Journal of Comparative Neurology*, vol. 351, no. 4, pp. 585–601, 1995.

[38] J. L. Moreau and H. L. Fields, "Evidence for GABA involvement in midbrain control of medullary neurons that modulate nociceptive transmission," *Brain Research*, vol. 397, no. 1, pp. 37–46, 1986.

[39] J. Sandkuhler, E. Willmann, and Q. G. Fu, "Blockade of GABA(A) receptors in the midbrain periaqueductal grey abolishes nociceptive spinal dorsal horn neuronal activity," *European Journal of Pharmacology*, vol. 160, no. 1, pp. 163–166, 1989.

[40] E. Carstens, M. Hartung, B. Stelzer, and M. Zimmermann, "Suppression of a hind limb flexion withdrawal reflex by microinjection of glutamate or morphine into the periaqueductal gray in the rat," *Pain*, vol. 43, no. 1, pp. 105–112, 1990.

[41] A. Depaulis, M. M. Morgan, and J. C. Liebeskind, "GABAergic modulation of the analgesic effects of morphine microinjected in the ventral periaqueductal gray matter of the rat," *Brain Research*, vol. 436, no. 2, pp. 223–228, 1987.

[42] M. A. Martins, A. P. Carobrez, and C. R. Tonussi, "Activation of dorsal periaqueductal gray by glycine produces long lasting hyponociception in rats without overt defensive behaviors," *Life Sciences*, vol. 83, no. 3-4, pp. 118–121, 2008.

[43] E. Palazzo, F. Guida, A. Migliozzi et al., "Intraperiaqueductal gray glycine and D-serine exert dual effects on rostral ventromedial medulla On- and Off-cell activity and thermoceptive threshold in the rat," *Journal of Neurophysiology*, vol. 102, no. 6, pp. 3169–3179, 2009.

[44] V. de Novellis, I. Marabese, R. Uliano et al., "Type I and II metabotropic glutamate receptors modulate periaqueductal grey glycine release: interaction between mGlu2/3 and A1 adenosine receptors," *Neuropharmacology*, vol. 43, no. 7, pp. 1061–1069, 2002.

[45] M. Nakamura and I. S. Jang, "Presynaptic nicotinic acetylcholine receptors enhance GABAergic synaptic transmission in rat periaqueductal gray neurons," *European Journal of Pharmacology*, vol. 640, no. 1–3, pp. 178–184, 2010.

[46] B. I. Min, C. J. Kim, J. S. Rhee, and N. Akaike, "Modulation of glycine-induced chloride current in acutely dissociated rat periaqueductal gray neurons by μ-opioid agonist, DAGO," *Brain Research*, vol. 734, no. 1-2, pp. 72–78, 1996.